HYDROCORTISONE INDUCES AORTIC RUPTURE IN INBRED BLOTCHY MICE. IMPLICATIONS FOR ABDOMINAL AORTIC ANEURYSMAL DISEASE IN HUMANS.

Edward Bruce Savage

1985

Permission for photocopying or microfilming of "

Hydrocortisone Induces Aortic Rupture in Inbred Blotchy Mice.
(title of thesis)
Implications for Abdominal Aortic Aneurysmal Disease in Humans"

for the purpose of individual scholarly consultation or reference is hereby granted by the author. This permission is not to be interpreted as affecting publication of this work, or otherwise placing it in the public domain, and the author reserves all rights of ownership guaranteed under common law protection of unpublished manuscripts.

Edward B. Savage
(Signature of author)

Edward B. Savage
(Printed name)

3-6-85
(Date)

Hydrocortisone Induces Aortic Rupture in Inbred Blotchy Mice. Implications for Abdominal Aortic Aneurysmal Disease in Humans.

A Thesis Submitted to the Faculty of the Yale University School of Medicine in Partial Fulfillment of the Requirements of the Degree of Doctor of Medicine

Edward Bruce Savage

1985

TABLE OF CONTENTS

Acknowledgements

B Pratt, K Sniderman, A Hudson for technical assistance; M. David Tilson for allowing me to come into his lab, providing financial, technical and intellectual assistance, moral support and the freedom to work as an independent investigator; my parents for believing I had the potential to succeed.

Dedicated to my wife Susan.

Abstract

Hydrocortisone Induces Aortic Rupture in Inbred Blotchy Mice. Implications for Abdominal Aortic Aneurysmal Disease in Humans.

Edward Bruce Savage
1985

Blotchy female and Black female mice from our Blotchy mouse breeding colony and C57BL/6J Black female mice from an independent colony were treated with hydrocortisone in drinking water at concentrations of 0.045, 0.15, 0.30, and/or 0.45 mg/ml. Seventy percent of the Blotchy females and 64% of the Black females from our colony died of intrathoracic hemorrhage with a mean survival of 15.6 and 15.5 days. Only 20% of the mice from the independent colony died of rupture, instead many died with bowel necrosis or of indeterminate cause with a mean survival of 19 days.
A dose-response curve for the response to hydrocortisone by Blotchy females is presented. From the experimental data the following conclusions are drawn:
(1) Expression of the genetic predisposition of Blotchy mice to develop aortic aneurysms is promoted by hydrocortisone. This effect is dose dependent and saturable.
(2) The Blotchy trait of aneurysm formation may be determined by more than one genetic locus.

INTRODUCTION

For many years nonmycotic, nonsyphlitic aortic aneurysms were attributed to the destructive effects of atherosclerosis eroding the molecular support of the aortic wall. This has since been called into question with evidence emerging to suggest that there may be a genetic predisposition, caused by a defect in connective tissue synthesis, that allows the development of aneurysms. Recently introduced into the surgical literature is the Blotchy mouse, an animal model for the X-linked genetic transmission of a trait that predisposes the animal to developing an aortic aneurysm. The Blotchy mouse is named for its pattern of coat coloring, also an X-linked trait. Hemizygous affected males are pale and most develop aortic aneurysms; heterozygous females have a patchy coat with areas of light and dark hair and some develop aneurysms; normal mice are black and do not develop aneurysms. These mice are thought to have an abnormality of copper metabolism responsible for both of these phenotypic traits.

Judah Folkman, independently examining an angiogenesis regimen of heparin and hydrocortisone in mice (39) noted that some of the mice turned pale and died from aortic rupture (Folkman, personal communication). We thought this might be an effect of heparin, acting as a copper scavenger, on copper metabolism thus creating a situation similar to that existing in the Blotchy and copper deprived mouse. Thus we set out to

examine the effects that this regimen would have on the Blotchy females hoping to see a change in coat color. To our surprise, in two weeks all of the mice were dead, many of them from aortic rupture. At this point emphasis was focused on hydrocortisone as the etiologic agent and the peculiar predisposition of Blotchy mice to develop aneurysms. Herein are contained results of these studies and conclusions drawn from these results, that:

(1) Expression of the genetic predisposition of Blotchy mice to develop aortic aneurysms is promoted by hydrocortisone. This effect is dose dependent and saturable.

(2) That Blotchy mice may actually carry two separate genetic traits which combine to give the Blotchy phenotype; the classic copper metabolism alteration of the mottled locus and a second, undefined locus which further weakens connective tissue structure.

Aortic aneurysms can be saccular, fusiform or dissecting. Traditional dogma ascribes fusiform abdominal aortic aneurysms to the destructive effects of atherosclerosis and hypertension. In the 1984 edition of Braunwald's book on heart disease the author reports that,

> "Abdominal aortic aneurysms arise in some areas of dense atherosclerosis. The atherosclerotic process erodes the aortic wall, destroying the medial elastic elements." (12)

The basic theory, that is an extension of this statement, is that occlusive disease and aneurysmal disease are different responses to the destructive and reparative processes of atherosclerotic scarring (107). That atherosclerosis is the sole cause of these aneurysms has been questioned in recent years on the basis of several observations by perceptive surgeons. In 1978 a paper was published defining two populations of patients with aorto-iliac disease. The first group which comprised 15% of the population was called the "stenosing" type because the lesions were associated with generalized peripheral stenosing arteriosclerosis. The other group called the "dilating" type, included 85% of the cases reported and demonstrated an abdominal aortic aneurysm with accompanying central and peripheral arterial dilatation (73). A study quantitating this dilatation reported that vessels

remote from the aortic aneurysms were 40-48% larger than those of occlusive controls (99). These two groups were further defined by associated vascular problems. The "stenosing" group had many signs and symptoms of generalized occlusive disease including carotid and coronary artery disease and peripheral vascular disease. Very few had associated aneurysms of other vessels. Patients in the "dilating" group had fewer occlusive problems but quite often had associated aneurysms of other vessels (46). Thus the concept of a population with an inherent predisposition to aneurysmal disease began to emerge and the role of atherosclerosis became less certain.

The concept of an inherent predisposition to aneurysmal disease was advanced in 1980 when a review of 50 consecutive aorto-iliac bifurcation grafts for aneurysmal or occlusive disease further defined these two populations; that with the aneurysm (dilating) was older (67 years v. 55 years) and showed a statistically significant difference in incidence between males and females. Eighty-eight percent of the aneurysms occurred in males whereas the sex distribution in occlusive (stenosing) disease was approximately equal (103). Further advancing these findings have been preliminary reports of families with more than one member developing an aortic aneurysm, with a rate of incidence much greater than would be predicted based on random occurrence rates for the general population (21, 102). The strong male predominence noted above suggests X-linkage may be involved in the

transfer of this trait. As family histories are accumulated the mode of transmission becomes less clear. A study of fifty families with more than one affected member reports many patterns of transmission suggesting an autosomal dominant inheritance with sex limitation to explain male preference (101). The true mode of transmission has yet to be defined, nevertheless this work implies that the etiology of abdominal aortic aneurysms may be a defective gene product.

Although the morphology of an aortic dissection is different from that of a fusiform aneurysm, its association with Marfan's Syndrome is a good model of a genetic trait causing aortic wall weakness. Patients with Marfan's Syndrome have a greater tendency to dissection than the general population (82). This tendency is due to a genetically transferred defect in collagen crosslinkage secondary to a defect in the structure of the collagen molecule. This fact lends credence to the theory that there is a genetically coded structural defect that predisposes to aortic dissection and that another defect may exist that predisposes to the development of abdominal aortic aneurysms.

Work in the past has illustrated other factors that contribute to the development of abdominal aortic aneurysms in man. Studies of the comparative anatomy of the aorta have shown that the number of elastic lamina in a particular segment is proportional to the stress normally applied to it (44). The human aorta deviates from the pattern consistantly

noted in other mammalian species. If it followed this pattern the abdominal portion of the human aorta would have forty elastic lamina to provide the necessary tensile strength to withstand normal systemic pressure; instead it has only twenty-nine layers (106). As a result of this each elastic layer in the human abdominal aorta must withstand proportionally greater stress than layers in other parts of the aorta. This observation can account for the preferential development of an aortic aneurysm in the abdomen but cannot explain the differential predisposition within the human species.

Experiments quantitating the content of structural molecules in the wall of an abdominal aortic aneurysm have shown that the percent of scleroprotein (collagen and elastin) is less than and the ratio of collagen to elastin is greater than that of the non-aneurysmal infrarenal aorta (90, 97). Based on these observations a logical assumption is that the trait for aneurysm development may involve a defect in the metabolism of the structural molecules of the vessel wall: Either a deficiency in synthesis or increased turnover of this framework. The concept of increased turnover will be discussed here and that of a defect in synthesis in the section on Collagen and Elastin.

Studies have been done assessing the activities of elastase and collagenase in the aortic wall from patients with and without aneurysms. The first compared eleven patients with aneurysmal lesions and five patients with

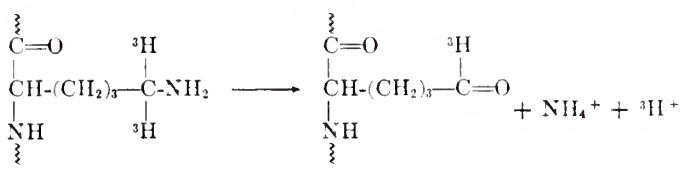
COLLAGEN AND ELASTIN IN AORTIC STRUCTURE

The two major structural proteins of the aortic media are collagen and elastin. Structural collagen is a fibril formed from monomers secreted by smooth muscle cells. These monomers, secreted as procollagen, after cleavage by extracellular enzymes spontaneously polymerize to form collagen fibers. This is a weak, acid soluble form in which the monomers are held together only by physical forces. The collagen fiber gains strength with the spontaneous formation of covalent crosslinks between the monomers (36). Elastin is synthesized in a similar fashion, secreted as tropoelastin monomers which are then crosslinked to form macromolecules (80).

The role of each of these macromolecules is unique. In an experiment to study the role of each molecule in maintaining vessel integrity, canine carotid arteries were treated with collagenase or elastase and then distended with increasing pressure to 150mmHg. Vessels treated with elastase to examine the role of collagen dilated markedly but did not rupture. All vessels treated with collagenase to examine elastin dilated very little then ruptured (31). From this result the author concluded that intact collagen is necessary to maintain the structural integrity of the vessel wall. Collagen performs this role by forming long fibrils

interconnecting the various components of the vessel wall and encompassing the smooth muscles and elastic lamina with a fine reticular meshwork (4, 44, 45). Elastin lends compliance to the aortic wall. It exists in a globular form radiating microfibrils to surrounding structures. This globule contains many hydrophobic amino acids which allow the chain to recoil to its original form when tension is released (80).

All this aside, neither molecule could function without being linked to its neighbor. Lysyl Oxidase plays a key role in this aspect of scleroprotein synthesis. This copper metalloenzyme appears to be the only enzyme necessary for the synthesis of crosslinks. It converts the amino side chains of lysine and hydroxylysine residues in elastin and collagen monomers to the aldehydes allysine and hydroxyallysine (37, 79). All crosslink reactions after this occur spontaneously (37).



$^3H^+ + H_2O \rightleftharpoons {}^3HHO + H^+$

FIG. 1.—Release of tritium during allysine formation.

Figure 1
(79)

The major crosslinks of elastin are desmosine and isodesmosine, each formed from three allysines and one lysine (80). Figure 2 (89)

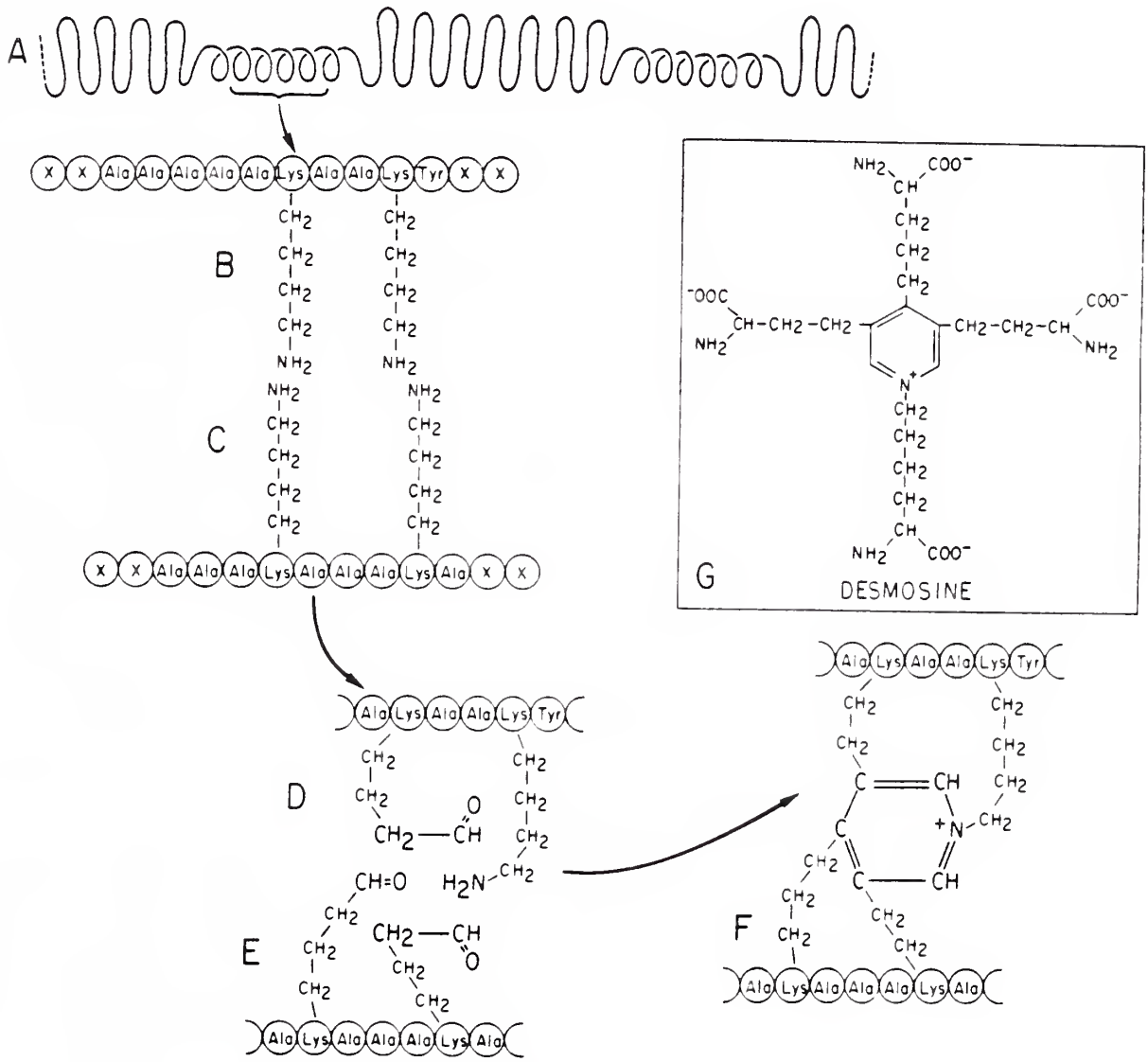


Figure 1. Cross-Linking of Soluble Elastin into Insoluble Elastin.

In A, the polypeptide chain of soluble elastin (tropoelastin) consists of elastic areas (large loops) alternating with inelastic areas (small loops). The elastic areas are composed largely of hydrophobic (nonpolar) amino acid residues, such as glycine, alanine, proline, and valine. The inelastic areas are composed of polyalanine sequences with interspersed lysine residues. These areas are potential cross-link sites and possibly exist as alpha helixes.

B and C show the apposition of two cross-linking sites on different chains, with the probable positions of the lysine side chains before the oxidative removal of the epsilon amino groups. In B, two alanine residues separate the two lysine residues; in C there are three alanines.

In D and E, aldehydes have replaced three of the four epsilon amino groups of the lysine side chains. The chains are folded to indicate their probable contribution to the desmosine ring structure.

In F, the pyridinium ring of desmosine has been formed with its resonating double bonds.

G shows a desmosine molecule free from peptide linkages to the elastin polypeptide chains. With isodesmosine, the lysine-derived side chain opposite the nitrogen (para) is moved to the ortho position.

Collagen has two major types of crosslinks. The first are "reducible" with sodium borohydride and include many reaction products of various interactions between lysine, allysine, hydroxylysine, hydroxyallysine and histidine. The second type are "non-reducible" crosslinks recently identified as hydroxypyridinoline and lysyl pyridinoline. Each a 3-hydroxypyridium ring; the former from 3 hydroxylysine residues, the latter from two hydroxylysine and one lysine residues. These are thought to derive from further addition of lysine or hydroxylysine components to certain "reducible" crosslinks but the true mechanism of formation has not been established yet (36, 37, 42, 43).

Figure 3
(37)

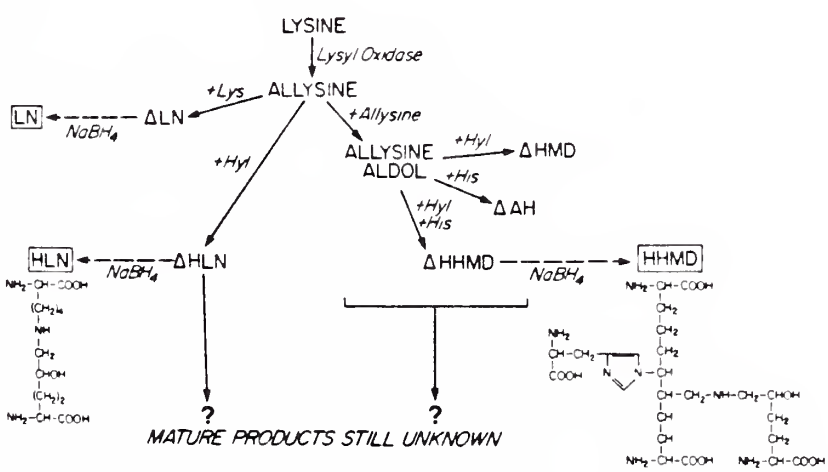


Figure 1 Scheme outlining one of two routes of cross-linking in collagen: Allysine-based cross-links. LN, lysinonorleucine; HLN, hydroxylysinonorleucine; HMD, hydroxymerodesmosine; AH, aldolhistidine; HHMD, histidinohydroxymerodesmosine. The prefix Δ for dehydro signifies the natural, aldimine forms of the various compounds.

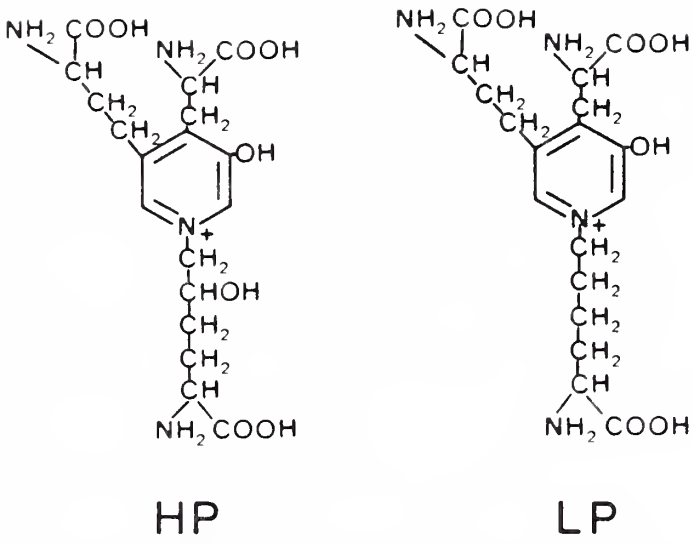
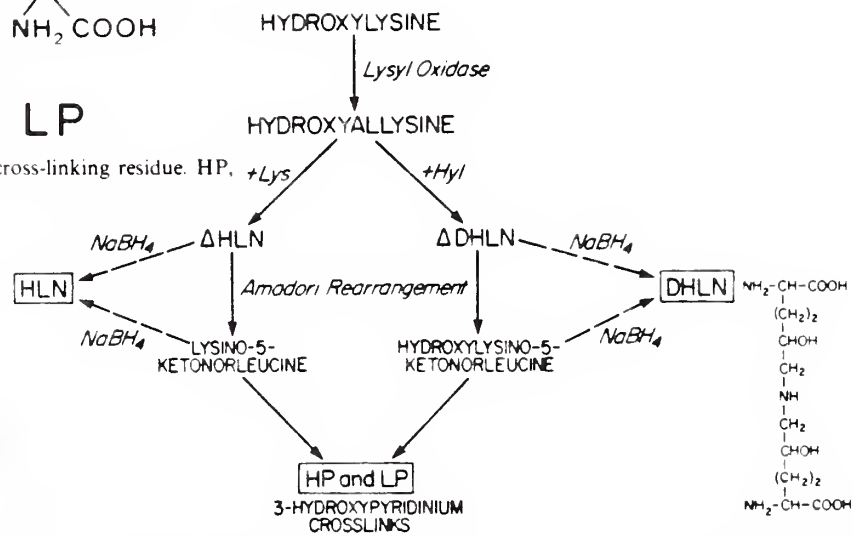


Figure 4
(37)

3 Structures of the two forms of 3-hydroxypyridinium cross-linking residue. HP, xylysyl pyridinoline; LP, lysyl pyridinoline.



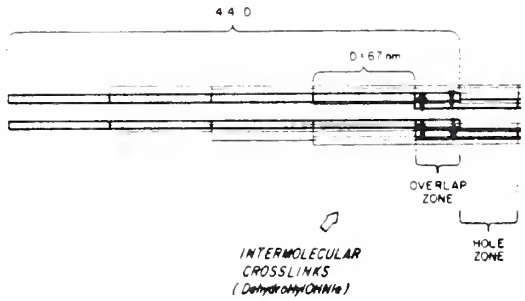


Figure 2 Scheme outlining one of two routes of cross-linking in collagen: Hydroxyallysine-based cross-links. DHLN, dihydroxylysinonorleucine; HP, hydroxylysyl pyridinoline, LP, lysyl pyridinoline. The prefix Δ for dehydro, signifies the natural, aldimine forms of the various compounds.

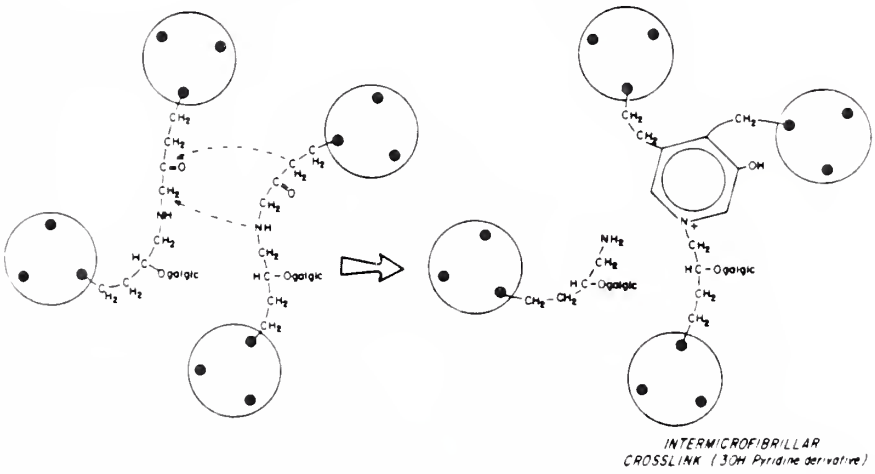


Figure 5
(37)

Figure 6
(36)

Fig. 4. Proposed mechanism (*100*) for the formation of the trivalent 3-hydroxypyridinium cross-links of collagen by interaction of two divalent cross-links. The dehydro-dihydroxylysinonorleucines are shown glycosylated and in their ketoamine configuration, their predominant form in collagens of bone and cartilage. Other reaction mechanisms are possible, although the one indicated is favored, and the product agrees with the 1,4,5 side-chained structure suggested by the data of Fujimoto (99). The upper molecular diagram shows how difunctional cross-links in adjacent five-stranded microfibrils can be spatially in close apposition, favoring their chemical interaction. Single-stranded filaments of head-to-tail overlapping molecules could interact equally effectively wherever they are registered as shown here.

Previously mentioned was the theory that a defect in scleroprotein metabolism may predispose to aortic aneurysm development. Increased turnover has been discussed. One can postulate, based on the proposed roles for collagen and elastin, that deficient synthesis of or synthesis of defective molecules of either of these components but especially of collagen would severely compromise the tensile strength of the vessel media. In a study of patients with ruptured cerebral aneurysms the ruptured vessels in eleven of seventeen patients were Type III collagen deficient (74). If a similar type of defect exists in the human abdominal aorta, which is already weakened by the deficiency of medial layers it might become too weak to withstand the stress of blood pressure, especially in the presence of systemic hypertension.

Several human syndromes have been described characterized by various forms of connective tissue weakness. Marfan's Syndrome and Ehlers-Danlos Syndrome comprise a group of disorders characterized by defects in structural protein, many caused by defective crosslinkage (37). The defect in Ehlers-Danlos Syndrome Type V is thought to be due to defective activity of lysyl oxidase leading to decreased formation of stable "non-reducible" crosslinks (30). In one form of Marfan's Syndrome there is a normal complement of Types I and III collagen, normal lysyl oxidase activity and normal to high levels of reactive aldehydes but a fifty

percent lower than normal density of 3-hydroxypyridinium crosslinks. The defect is thought to be inherent to the monomer structure or processing that prevents spontaneous crosslinkage; perhaps by causing intermolecular misalignment (11). These two types described have different molecular defects and mechanisms leading to a similar results; a defect in the formation of crosslinks. Both of these diseases are also a part of a spectrum comprising many phenotypes with as yet undefined molecular defects. Noting the variable effects that one mutation can have on connective tissue structure and strength and the various genetic defects that can effect connective tissue, it is reasonable to assume that the trait for development of aortic aneurysms may be another expression of a defect in connective tissue synthesis.

BLOTCHY MICE AND OTHER ANIMAL MODELS OF AORTIC ANEURYSM

There are a few animal models of aortic aneurysm including the Broad-breasted White turkey (10, 47) and the Blotchy mouse (3). Blotchy is the member of a family of mice called Mottled. Mottled includes mice that have in common a variation in coat color that is genetically transmitted in an X-linked pattern. Normal males (+/Y) and females (+/+) are black. Hemizygous males (Mo/Y) are pale with little pigmentation. Heterozygous females (Mo/+) are intermediate demonstrating a pattern of transverse bands of lighter and darker pigmentation. There are five types of mice thought to express different alleles at the mottled locus: Blotchy, Brindled, Dappled, Viable Brindled, and Tortoise. Each of these has in common coat color but each expresses independent abnormalities (93). Brindled hemizygous males (Br/Y) rarely survive more than fourteen days. This is related to a deficiency in brain norepinephrine caused by deficient activity of dopamine beta-hydroxylase (56, 57). Blotchy mice are notable for the tendency of hemizygous males (Blo/Y) to die from a ruptured aortic aneurysm, and to have decreased skin tensile strength and joint disease (33, 37, 84, 92). Ninety-eight percent of Blotchy hemizygous males develop aortic aneurysms. The aneurysms usually rupture after 150 days of age. The mice have a median life span of 200 days with ninety percent dead by one year (93). Data on the

occurrence of aneurysms in other mice is presented in Table I.

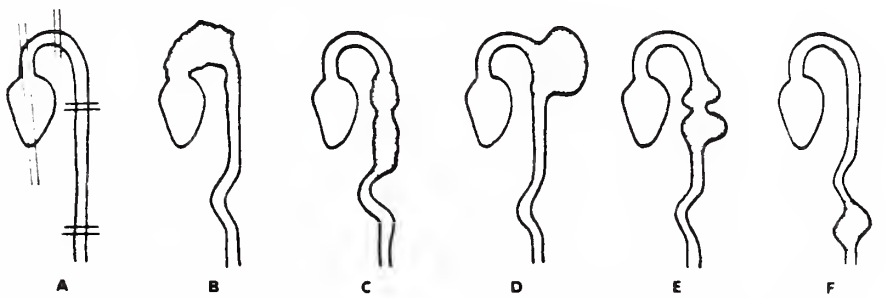
Table I

Mouse	Aortic Lesion (percent)		
	Normal	Aneurysm	S-curve
Blo/+	63	32	5
Blo/Blo	15	85	
Blo/Y	2	98	
Blo/Br	100		
Br/+	100		
Br/Y	100		
Dap/+	90	4	6
To/+	58	25	17
To/Y	19	81	

S-curve refers to aortic ectasia (93)

Most of the aneurysms are saccular or fusiform. They arise in the aortic arch or the proximal portion of the descending aorta. At times they are multiple (3).

Figure 7 (3)



TEXT-FIG 1—Diagramatic illustration of the many gross appearances of aortic aneurysms.

These defects have been attributed to a deficiency of lysine derived crosslinks in collagen and elastin secondary to a lack of lysine derived aldehydes synthesized by lysyl oxidase (84). In quantitative studies skin extracts from Blotchy mice show decreased lysyl oxidase activity. Hemizygous males (Blo/Y) have activity that is only 5-30% of littermate controls (85). Heterozygous females (Blo/+) have activity greater than fifty percent of controls. This activity parallels the severity of the cross-link defect and the amount of acid soluble collagen pointing to the decreased activity of lysyl oxidase as the cause of the structural defect in connective tissue of Blotchy mice (85). The decreased lysyl oxidase activity could be a result of (a) an absolute decrease in tissue levels of lysyl oxidase due to decreased synthesis or increased breakdown, (b) synthesis of and altered enzyme with a higher Km, or (c) adequate enzyme synthesis with inadequate availability of the copper cofactor. Most research into the nature of this defect has focused on the copper as a cofactor of lysyl oxidase and the abnormalities of copper metabolism in mottled mice (55).

The defects in Brindled and Blotchy mice are those most studied of the Mottled mice. General agreement is that there is a defect in copper metabolism and that its effects are mediated by the deficient activities of copper dependent enzymes. Table II lists some of these enzymes and their activities.

Table II

Copper Metalloenzymes	Functional role
Cytochrome C oxidase	Electron transport chain
Superoxide dismutase	Free radical detoxification
Dopamine B hydroxylase	Catecholamine production
Lysyl oxidase	X-link collagen and elastin
Tyrosinase	Melanin production
Ceruloplasmin	Ferroxidase, Cu transport

(16)

Hemizygous Brindled mice (Br/Y) are notable for reduced growth rate, reduced pigment synthesis, abnormal hair structure, neurological abnormalities including ataxia and tremors and reduced viability (death at about 14 days) (54). These mice have a defect in copper metabolism characterized

by deficiencies of copper in some organs and concentration to excess in others. Specifically this includes impaired intestinal copper absorption characterized by normal epithelial uptake, excess mucosal accumulation and decreased efflux; deficient hepatic copper uptake; decreased brain copper levels and renal copper concentration to levels in excess of normal (35). Brindled mice are considered to be a model for the human Menkes' Kinky Hair Syndrome (15). This is an X-linked disorder with symptoms that include abnormal hair growth, depigmentation, arterial degeneration, growth retardation, progressive neurological degeneration and death in the first year of life (6, 16). These patients have a similar defect in copper metabolism with defective gut copper absorption and mucosal, renal, pancreatic, osseous, muscular and dermal copper excess and a deficiency of copper in liver and all other tissues (16, 80).

Hemizygous Blotchy male mice (Blo/Y) display a phenotype similar to Brindled mice including poor growth, abnormal hair structure and depigmentation. However they differ in their significantly longer life span and development of and death from aortic aneurysms (29). In the hemizygous Blotchy male (Blo/Y) gut copper absorption is 64% and hepatic copper content is 56% that of normal mice, but copper accumulation in fibroblasts is 500% that of normal mice (95). In experiments feeding Blotchy mice radioactive copper (Cu64) the hemizygous males (Blo/Y) demonstrated increased accumulation of copper in kidney, lung, connective tissue,

skin and duodenum. Heterozygous females (Blo/+) showed similar results with increased kidney, skin and connective tissue copper content but no increased duodenal copper accumulation. However both males and females had decreased liver copper accumulation (77). All this points to what is considered a similar defect in copper metabolism in Brindled and Blotchy mice and Menkes' Syndrome. The fact that each mouse has a distinct phenotype suggests that Brindled and Blotchy may be separate alleles of the same genetic locus.

The etiology of this defect in copper metabolism with deficient gut absorption and selective cellular concentration and deficiency has defied definition for many years. In culture, skin fibroblasts from patients with Menkes' Syndrome show increased and excessive accumulation of radioactive copper. When these cells are placed in a non-radioactive medium the release of radioactive copper is slower and less complete than normal controls (8). Most of this excess copper is bound to metallothionein(MT) which is the major cytosolic storage protein for copper and is also present in excess in these cells. Thus the defect in some organs may involve some aspect of cellular copper metabolism where copper is taken up in excess by cells but unavailable for use by cellular enzymes. As outlined in a recent review possible causes of this are (1) an altered MT with increased affinity, (2) increased stability of MT, (3) a regulatory defect with increased MT synthesis, (4) an altered intracellular copper transport protein or (5) a defect of copper efflux or

utilization with secondary MT synthesis (15). Most theories concentrated on a defective MT. However the MT-I gene has since been mapped to mouse chromosome 8, precluding X-linked transmission and pointing to a defect independent of MT and yet to be defined (25).

Based on the assumption that there is a primary defect of copper metabolism, how does this convey the phenotypes of Blotchy and Brindled? Coat color, a common characteristic of both of these phenotypes is mediated by Tyrosinase. Tyrosinase is the first enzyme in a multistep pathway of conversion of tyrosine to melanin. It requires a copper cofactor for activity and phenylthiourea or sodium diethylthiocarbamate inhibit its activity by combining with the copper in the enzyme (63). The coat of C57BL/6J mice fed a copper deficient diet for four weeks will change from intense black to light brown in regrown hairs demonstrating this enzyme's acute need for copper for normal activity. Analysis of the tyrosinase from these copper deprived mice and mottled mice show similar properties including similar results on dopa-incubated electropherograms and that there is synthesis of considerable amounts of inactive apoenzyme (50). Copper supplementation to brindled males, blotchy males and heterozygous blotchy females leads to darker, if not black new hair growth (54; Savage EB, Tilson MD, Gertler J unpublished observations). Based on these observations the coat color characteristics can be related to the deficient activity of tyrosinase and lack of melanin synthesis in

individual melanocytes. Thus the hemizygous males (Blo/Y) are pale because all of their melanocytes express the cellular defect of copper metabolism. In the heterozygous female (Blo/+), consistant with the X-linked nature of this defect, the Lyon Hypothesis can be invoked to explain the presence of normal and abnormal melanocytes giving the characteristic mosaic of light and dark hairs.

Dopamine-B-Hydroxylase catalyzes the conversion of dopamine to norepinephrine. It too is a copper dependent enzyme and copper chelaters will inhibit its activity (23). Brindled mice have decreased central and peripheral norepinephrine synthesis with accompanying accumulation of the precursors tyrosine and dopamine. This is thought to contribute to the neurological defects and early death of brindled males (56, 57). When supplemented with copper these mice live beyond ninety days of age (untreated they die at fourteen) and have higher levels of brain norepinephrine and increased DBH activity (54, 105).

The most notable deficiency of a copper metalloenzyme activity expressed in the Blotchy mouse is that of lysyl oxidase with the resultant formation of aortic aneurysms by mechanisms previously described. Young chicks and pigs raised on copper deficient diets die from structural lesions of the major arteries with conspicuous defects of the elastic membrane and decreased mechanical strength of affected vessels (20, 91). Lathyrogens such as Beta-aminopropionitrile cause similar lesions by inhibiting the

activity of lysyl oxidase (20). Studies of both Brindled (Br/Y) and Blotchy (Blo/Y) male mouse skin shows lysyl oxidase activity to be only 5 to 30 percent that of littermate controls. Heterozygous Blotchy females (Blo/+) have skin lysyl oxidase activity that is greater than 50 percent that of controls (85). In humans, fibroblasts from Menkes' Syndrome patients and the proposed Ehlers-Danlos Type IX patients have decreased lysyl oxidase activity with increased intracellular copper levels pointing to an intracellular defect of copper metabolism that makes intracellular copper unavailable to lysyl oxidase (67, 78, 86). Copper supplementation of chicks previously fed a copper deficient diet will markedly increase aortic lysyl oxidase activity (48, 49). This has also been demonstrated IN VITRO with cells from copper deprived chick aortas. There is a 3-5 hour delay in the appearance of lysyl oxidase activity after the copper is added to the media and cyclohexamide, an inhibitor of protein synthesis, will block this response demonstrating that new synthesis is required for copper utilization (81). Of interest is that propanolol appears to enhance the activity of lysyl oxidase and in aneurysm prone turkeys will lead to increased tensile strength and decreased incidence of aortic rupture, an effect independent of its blood pressure and pulse rate lowering effect (16).

As noted previously, copper supplementation of Brindled and Blotchy mice and copper deprived animals will lead to the

increased activity of some copper metalloenzymes. However, treatment of Blotchy mice with copper which will be recalled leads to darker coat color, shows some interesting deviation from this pattern: (1)Skin lysyl oxidase activity of Blotchy mice does not respond as well to copper treatment as does that of Brindled mice and (2)cytochrome C oxidase activity which responded quite well to treatment in Brindled mice failed to respond in Blotchy mice (16). This coupled with the conspicuous absence of aneurysms in Brindled mice points to the possibility that the defects in Brindled and Blotchy may not be allelic or that Blotchy may have an additional separate mutation elsewhere predisposing to aneurysms that is compounded by the copper dependent defect.

> [Recall from Table I that Brindled males do not develop aneurysms. This is deceptive because they do not live long enough to develop aneurysms. For a more realistic comparison note that 32% of the heterozygous Blotchy females (Blo/+) and 85% of (Blo/Blo) females develop aneurysms whereas none of the Brindled females (Br/+) and none of the Brindled/Blotchy females (Blo/Br) do.]

As a possible explanation for this it has been speculated that the genes for lysyl oxidase may be closely linked to those for copper metabolism on the X-chromosome thus a single mutation may affect both genes, but the true relationship still remains a mystery (85, 95).

The exact relationship of copper to aneurysm formation in humans has not yet been defined. Aneurysm patients have a significantly higher serum ceruloplasmin and copper levels than normal controls (91). There are conflicting reports of liver and skin copper levels in aneurysm patients. One study reports depressed copper levels (98, 100) and another study reports elevated copper levels (2). Thus the final chapter on copper metabolism and aneurysms in humans is far from complete.

THE EFFECTS OF STEROIDS ON COLLAGEN AND VASCULAR TISSUES

Steroids of various forms and functions are widely used to treat a broad spectrum of illnesses. Yet these compounds are not used without adverse consequences. The toxic effects of prolonged therapy with glucocorticoids include electrolyte imbalance, hyperglycemia, glycosuria, increased infection susceptability, peptic ulcers, osteoporosis, skin atrophy, growth inhibition, psychosis, adrenal atrophy and lymphoid tissue regression (28). Some of these effects are related to decreases in absolute amounts of structural tissue (osteoporosis, dermal atrophy). Since 80% of the dry weight of skin is collagen, a loss of collagen must occur with dermal atrophy (9). There are many reports of weakening and loss of connective tissue composed of collagen associated with steroid use. Prolonged steroid use leads to bone loss and a significant increase in the number of fractures in asthma patients (1). In patients with Kawasaki's disease, 65% of those treated with steroids developed coronary artery aneurysms compared to untreated controls of whom only 20% developed aneurysms (59). There are four reported cases of rupture of the Achilles' tendon after treatment of intractable pain from tendonitis with local steroid injections (61, 68). These ruptures occurred after minor stress, and the tendons were grossly soft and degenerated in comparison to the normal interdigitated, shredded appearance

of a traumatic tendon rupture. In rabbits with corneal injuries high doses of steroids will prevent adequate repair (70). Finally, it is notable that during pregnancy, a state of increased physiological levels of steroid hormones, women have a higher incidence of aortic dissection and of acute rupture of saccular splenic artery aneurysms (82).

There are a few specific models of steroids precipitating aortic rupture in animals. Of 24 turkeys treated with a 24 milligram subcutaneous pellet of diethylstilbestrol, 14 (58.3%) were dead between two to five weeks after implantation of dissecting aortic rupture (5). In experiments with hamsters treated with: (a) Melengestrol acetate (a progestogen) 15 of 36 died within eight weeks, 11 of supravalvular aortic rupture (22); (b) desoxycorticosterone (a mineralocorticoid) and cortisol or cortisone developed dissecting aortic rupture without increases in blood pressure (40); (c) desoxycorticosterone and cortisol developed dissecting aortic rupture in 6 to 36 days (41); (d) cortisone acetate, 51 of 77 died in 26 to 162 days of massive hemorrhage, 26 with proven aortic dissection originating just above the aortic valve (96).

There are two factors that are potentially responsible for these observations: (a) Decreased tensile strength or (b) increased stress. In the case of aortic rupture the latter would take the form of increased systemic blood pressure. Hypertension has been produced in rats with cortisone acetate (62) and methyl prednisolone (65) and

excess adrenal steroids are thought to produce the hypertension associated with Cushing's Syndrome (64).

In support of reduced tensile strength are many studies showing major effects of steroids on the structural components of the vascular wall, for example: Treatment of aortic smooth muscle cells from humans with cortisol (58) and from cows with dexamethasone (71) caused marked growth retardation. Cortisol caused an increase in collagenolytic and proteolytic activity in the extracellular, extrafibrillar compartment of rat skin and in rat fibroblast cultures within four hours (53). Prednisolone caused increased collagenolytic activity in fogarty catheter induced pressure injuries of rabbit aortas (72).

The above are interesting observations about cellular growth and catabolism but the major effects of steroids are probably anti-anabolic and relatively specific to new collagen synthesis. The first observations that steroids had an anti-anabolic effect on collagen synthesis were made in rats that, when treated with cortisone, had decreased urinary hydroxyproline excretion (a direct marker of collagen turnover). When cortisone was administered for ten days before 14C-proline, the hydroxyproline excretion and urine 14C-hydroxyproline specific activity were both lower; when 14C-proline was given for 30 days before the cortisone there was a small decrease in hydroxyproline excretion but no change in urine 14C-hydroxyproline specific activity. From this result the investigator concluded that there was an

anti-anabolic action of cortisone on the formation of new soluble cortisone but no effect on the catabolism of mature insoluble collagen (60, 94). In other studies it has been shown that glucocorticoids decrease the activity of lysyl oxidase and/or prolyl hydroxylase in a dose dependent manner in rat skin (7, 24, 27, 75), liver (26), and chick-embryo tendon cells (76). The decreased activities of these enzymes were thought to be one cause of the decreased collagen synthesis. However if this were the major cause then one would expect this to result in an increased ratio of proline to hydroxyproline in newly synthesized collagen. Steroids have not been shown to change this ratio from that of untreated tissue so the effect on collagen synthesis probably occurs prior to the activity of these enzymes on the collagen molecule (24).

There have been many models used to study the actual effects of steroids on collagen synthesis. Most studies report a dose dependent selective inhibition of collagen synthesis; selective because the steroids will suppress both non-collagen protein and collagen synthesis but will suppress the latter to a greater degree (28, 66, 75, 87, 104). A few studies have reported stimulation of collagen synthesis by steroids. In one of these the stimulatory effect was only noted in the log phase of cell growth; in the confluent phase there was a steroid dependent decrease in collagen synthesis (69). In another study the stimulatory effect was noted after five hours of treatment (32). Subsequent studies

report that often a stimulatory effect is noted in the first 24 hours of treatment but after that the steroid effects are generally depressive (17, 88). Thus most evidence points to a depressive effect of steroids on new collagen synthesis.

On a more basic level, glucocorticoids cause decreased uptake of precursor amino acids and decreased synthesis of procollagen mRNA (28). Lung and dermal polysomal mRNA isolated from triamcinolone treated rats showed a decrease in all mRNA production but a proportionally greater decrease in procollagen mRNA than non-procollagen protein mRNA (83). Decreased DNA synthesis has also been noted with steroid treatment (75, 104). A direct effect on DNA synthesis implies the presence of specific steroid receptors in the cells synthesizing collagen. In fact, the canine aorta has been shown to have highly specific receptors for estrogens, androgens and glucocorticoids that are concentrated in the media and adventitia (52).

In summary, there appears to be a very specific effect of glucocorticoids on structural proteins of the aorta causing structural weakness principally through a decrease in structural subunit synthesis.

MATERIALS AND METHODS

C57BL/6-J-Blotchy mice were obtained from an inbred colony maintained at Yale Medical School by M. David Tilson. Tetracycline, heparin (>140 USP U/mg), and hydrocortisone were purchased from Sigma Corporation (USA). Due to reported problems with infection (39) using a similar regimen of steroid treatment, precautions were taken including: (a) Using filter-frame cages, (b) wearing masks and gloves when handling mice, (c) autoclaving all food, drinking water, water bottles, cages and bedding, (d) changing cages twice a week and (e) supplementing drinking water with tetracycline as prophylaxis for infection. All pharmaceuticals were added to sterile distilled water and administered ad libitum in drinking water. The average mouse consumed 5-10 ml of drinking water per day. Adequate measurement of consumption was not possible on available equipment. The mice were fed the standard laboratory mouse food.

The experiments in this paper were performed in two stages.

STAGE I

Twenty Blotchy female mice (Blo/+) of various ages ranging from one month to one year were randomly assigned to four groups.

Group 1 Tetracycline

Group 2 Heparin

Group 3 Hydrocortisone, tetracycline

Group 4 Heparin, hydrocortisone, tetracycline

Dosages added to drinking water were based on previous work done by Folkman (1983): Tetracycline 750 mg/L, hydrocortisone 0.45 mg/ml, heparin >200 U/ml. Chemicals were replaced 6 days per week. Of the ten mice in groups 3 and 4, nine mice died and were autopsied and one was sacrificed on day 16 for angiography. One mouse in group 2 was sacrificed. Samples of heart, lung and aorta from the heart to the aortic bifurcation from selected animals were sent for histological staining.

STAGE II

After the discoveries of the first stage experimentation was concentrated on what were thought to be the aortic rupture inducing effects of hydrocortisone. Two types of experiments were performed. The first experiments compared the effect of different dosages of hydrocortisone on Blotchy Female (Blo/+) mice to derive a dose response curve. Dosages

used were 0.45, 0.30, 0.15 and 0.045 mg/ml. Data were derived using mean survival and length of survival to aortic rupture as a measure of drug effect. The second experiments compared the variable effects of a constant dose of hydrocortisone (0.45mg/ml) on Blotchy Female (Blo/+) and Black Female (+/+) mice from our breeding colony and Black Female (+/+) C57BL/6J mice from an independent colony at Jackson Laboratories. In all experiments treatment was continued until death or for twenty-one to thirty-two days, whichever came first. Selected animals were sacrificed for angiography and histological sectioning as noted in the results section.

Mice of various ages were used in different experiments but in all cases, except where noted, comparisons are made between age matched treatment groups.

Angiography was performed by manual instillation of 1:1 Renograffin:normal saline into the left ventricle. Mice alive at the time of angiography were anesthetized with sodium pentathal, a thoracotomy was performed and the dye was injected into the beating heart.

Histological slides were stained with Hematoxylin-Eosin and Elastin-Von Geison stains.

RESULTS

In general, the mice treated with 0.45 mg/ml of hydrocortisone came to have a bloated appearance, were sluggish at times moving very little, lost hair and on mice that had a patch of hair removed from their back there was no new hair growth. Their skin at autopsy was quite fragile, weak enough to be torn by hand. The coat color of a number of the black mice became grayer and some mice developed cataracts. Histological sections of the lungs of some mice showed a mononuclear cell infiltrate. Other sections revealed enlarged airspaces and ruptured alveolar septa.

STAGE I

Five mice were in each of the four treatment groups outlined in the Materials and Methods. Those treated with tetracycline or heparin alone were still alive at the end of the experiment on day 30 unless sacrificed for analysis. All mice treated with hydrocortisone and heparin/hydrocortisone were dead by day 15 except for one mouse in the latter group that was sacrificed for angiography on day 16. Results are summarized in Table III. Of the nine mice that died, six on autopsy were noted to have gross blood and clot in the thoracic cavity (see figure 8). The lungs were pale and

obviously not the source of the blood. The liver was pale consistent with massive hemorrhage. Three of these nine mice were too mangled on discovery for meaningful results on autopsy. Of these three, one (#31) died on day 1 suggesting an alternate cause of death. The other two (#22, #24) died on days 11 and 12 at the same time the other mice were dying suggesting that intrathoracic hemorrhage might have been the cause of their death, but this could not be verified. The tenth mouse, alive on day 16 was sacrificed for angiography. Two deaths were observed by the investigator: After being handled these two mice became agitated, took a few steps and suddenly died of what proved to be intrathoracic hemorrhage on autopsy.

The average length of survival before death of those mice that died in this experiment was 12.4 $\pm$ 1.8 days. The average length of treatment before proven aortic rupture was 12.7 $\pm$ 2.1 days (table IV). Using chi-square the statistical significance of aortic rupture was calculated for mice treated with hydrocortisone (table V). Calculations were performed comparing mice treated with hydrocortisone with or without heparin to an "untreated" group which received heparin or tetracycline to specifically evaluate the effect of hydrocortisone. If the death of the mangled mice 22 and 24 are not considered due to aortic rupture the calculated significance showed $P<0.05$. If mouse 31 which died on day 1 is excluded $P<0.01$. Finally, if the deaths of mice 22 and 24 are attributed to aortic rupture then $P<0.001$. These results

suggest that hydrocortisone in very high doses will precipitate weakening and eventual rupture of the aorta in heterozygous Blotchy female mice; in this experiment, within 15 days when added to drinking water at a concentration of 0.45 mg/ml. Subsequent experiments were designed to further examine and characterize this finding.

Anatomy of the Lesions

The mice were examined for gross lesions by autopsy and angiography; gross lesions were examined for histological abnormalities.

Autopsy examination of mice that had died of intrathoracic aortic rupture revealed no obvious abnormality until latex dye was injected into the left ventricle. The latex was noted to extravasate into the thoracic cavity, most commonly from a defect in the ascending aorta. One Black female mouse from a later experiment (Table X) which had an intact vascular system at the time of death demonstrated an intact saccular aneurysm of the aortic arch. More useful information was obtained from angiography. Angiography of a Blotchy Female mouse that had died with intrathoracic rupture demonstrated slight filling of the aorta to the level of the diaphragm with intrathoracic extrusion of the dye (see figure 9). Another Blotchy Female was sacrificed for angiography on day 16. After anesthesia and thoracotomy dye injected into the beating left ventricle demonstrated carotid ectasia and

dilatation and a prominent fusiform thoracoabdominal aneurysm with filling of the distal femoral arteries (see figure 10). Angiography of a Black female sacrificed on day 22 demonstrated a subdiaphragmatic fusiform aortic aneurysm, a saccular aneurysm distal to this with accompanying dilatation of the common carotid and subclavian arteries (see figure 11). Angiography of a mouse treated with 0.045mg/ml demonstrated the normal vasculature (Figure 12).

Histological sections were made of the heart, lungs, mediastinum and descending aorta from selected mice. A section of the ascending aorta (#9, EVG) reveals disruption of the elastic lamina of the media with outward displacement of the adventitia and extruded blood (figure 13). Another section (#23, EVG), again of the aorta, demonstrates dissection and rupture of the vessel wall with blood between the elastic lamina, extramural blood and outward displacement of the adventitia (figure 14).

Histological sections of the distal aorta were made on mouse #20 which had the fusiform thoracoabdominal aneurysm documented by angiography and on mouse #4 which had received only heparin and could serve as a control. No gross differences were noted on H&E and EVG preparations except that the aorta in mouse #20 was larger. A more rigid comparison cannot be made because of the age difference in these two mice.

TABLE III

Stage I

#	Age	Group	Day of Death	Autopsy results	Histology
31	1m	H/H	1	+++	---
9	4m	H/H	12	Gross blood & clot in thorax (see fig. 8)	Thoracic aorta - Gross perivascular blood - rupture of wall with extruded blood (see text) (see fig. 13)
24	2m	H	11	+++	---
22	12m	H	12	+++	---
23	2m	H/H	13	Gross blood & clot in thorax	Thoracic aorta - Gross dissection with intramural and extramural blood (see text) (see fig. 14)
29 ++	1m	H	13	Gross blood & clot in thorax	Perivascular blood in mediastinal fat
11 ++	3m	H	14	Gross blood & clot in thorax	---
13	3m	H/H	9	Gross blood & clot in thorax	---
7	4m	H	15	Gross blood & clot in thorax	---
20	12m	H/H	16	(sacrificed for angiography - see text)	Abdominal aorta- no gross abnormanlity
4	5m	Hep	18	(sacrificed) no gross abnormality	Abdominal aorta- no gross abnormality

Age m = months
Group H = Hydrocortisone alone
H/H = Hydrocortisone and heparin
Hep = Heparin
++ = died suddenly while handling
+++ = Destroyed beyond recognition on discovery

Table IV

Average length of survival of treated mice that died Stage I *

	Number	Mean(days)	SD
All deaths	8	12.4	1.8
Aortic rupture	6	12.7	2.1

* Excluding mouse 31 because of death on day one, excluding mouse 20 because it was alive on day 15.(see text)

Table V

Statistical data on aortic rupture Stage I

A. Occurrence of rupture by day 15 - death of 24 and 22 not considered due to aortic rupture. (see text)

1. Data includes mouse 31.

	hyd/hep	hep/tet	Total
Rupture	6	0	6
No rupture	4	10	14
Total	10	10	20
% rupture	60%	0%	

Significance of rupture in hyd/hep treated mice.
X c(1df)=5.95, P<0.05

2. Data excludes mouse 31.

	hyd/hep	hep/tet	Total
Rupture	6	0	6
No rupture	3	10	13
Total	9	10	19
% rupture	67%	0%	

Significance of rupture in hyd/hep treated mice.
X c(1df)=7.12, P<0.01

B. Occurrence of rupture by day 15 - death of 22 and 24 considered due to aortic rupture. Data includes mouse 31.

	hyd/hep	hep/tet	Total
Rupture	8	0	8
No rupture	2	10	12
Total	10	10	20
% rupture	80%	0%	

Significance of rupture in hyd/hep treated mice.
X c(1df)=11.0, P<0.001

hyd/hep-mice treated with hydrocortisone 0.45 mg/ml or hydrocortisone and heparin, both groups received tetracycline.

hep/tet-mice treated with heparin or tetracycline. Since we are evaluating the specific effects of hydrocortisone these can serve as "untreated" controls.

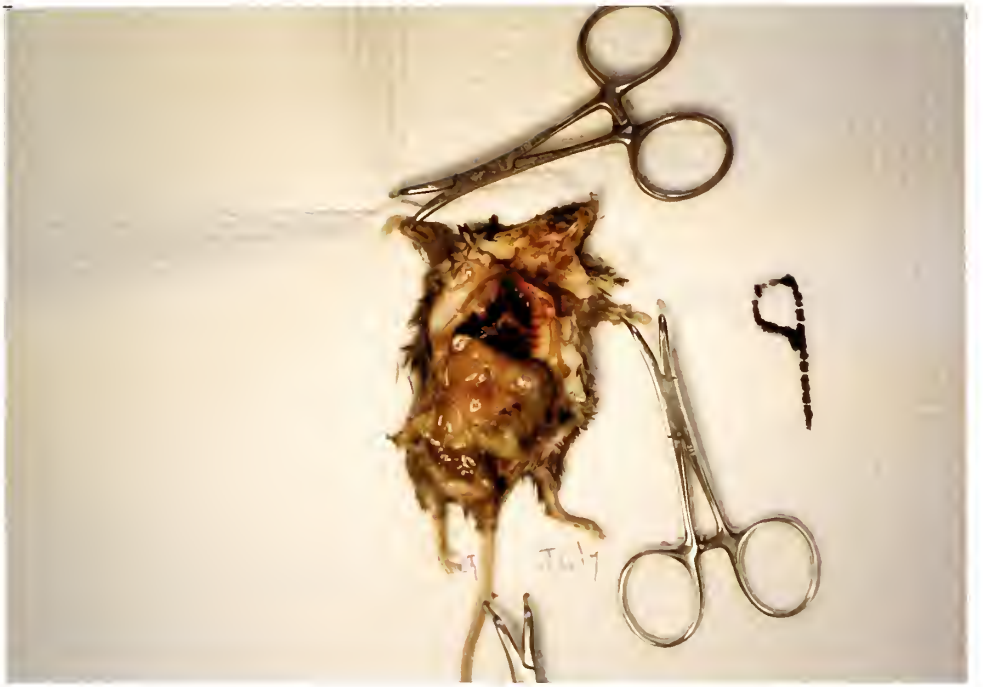


Figure 8 Autopsy findings with aortic rupture: Gross blood and clot in chest.

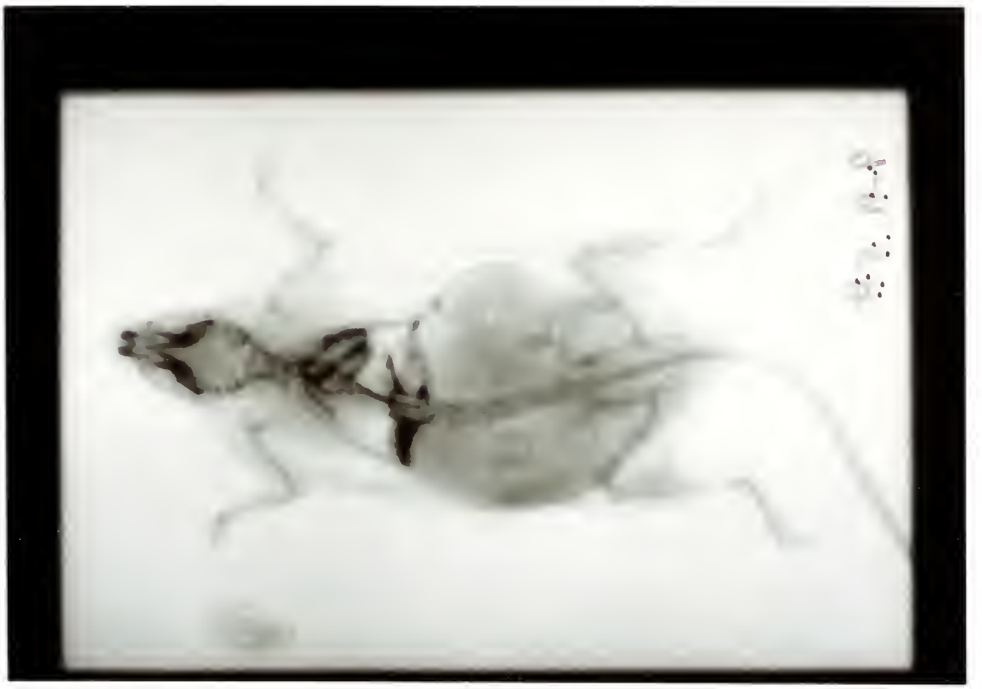


Figure 9 Angiogram of a mouse that died with rupture. Slight filling of the aorta to the diaphragm with intrathoracic extrusion of dye.

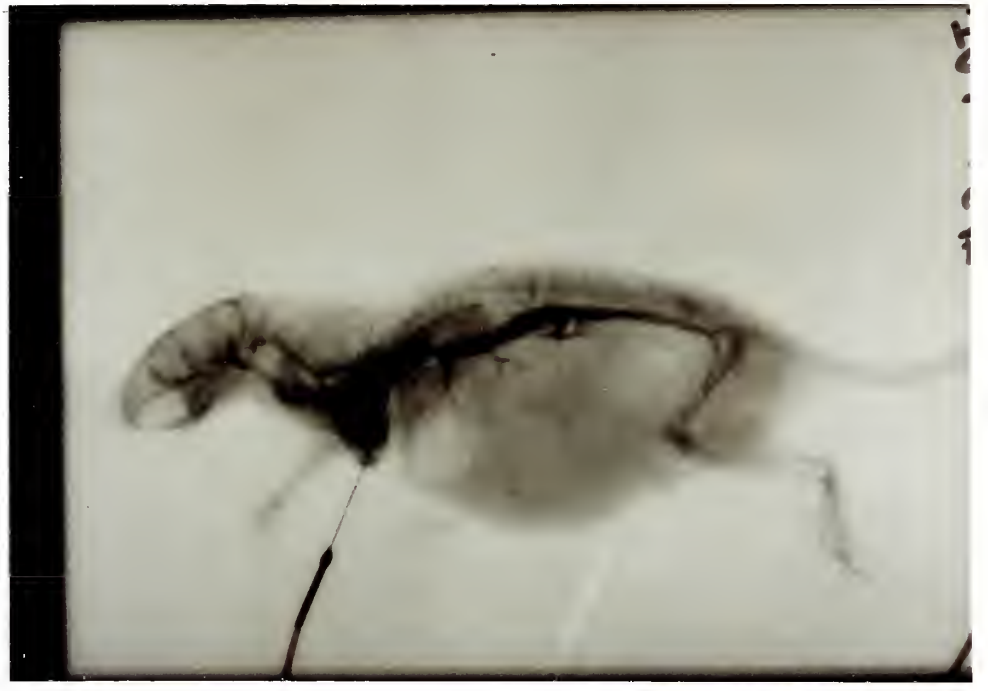


Figure 10 Blotchy female sacrificed for angiography on day 16. Note carotid ectasia and dilatation with a prominent fusiform thoraco-abdominal aneurysm and filling of the distal femoral arteries.

Figure 11A Angiography of a Black female sacrifice on day 22. Subdiaphragmatic fusiform aortic aneurysm, a saccular aneurysm distal to this and carotid and subclavian artery dilatation.
(AP view)

Figure 11B (lateral view)

Figure 12 Angiography of a Female treated with 0.045 mg/ml demonstrating the normal vasculature.

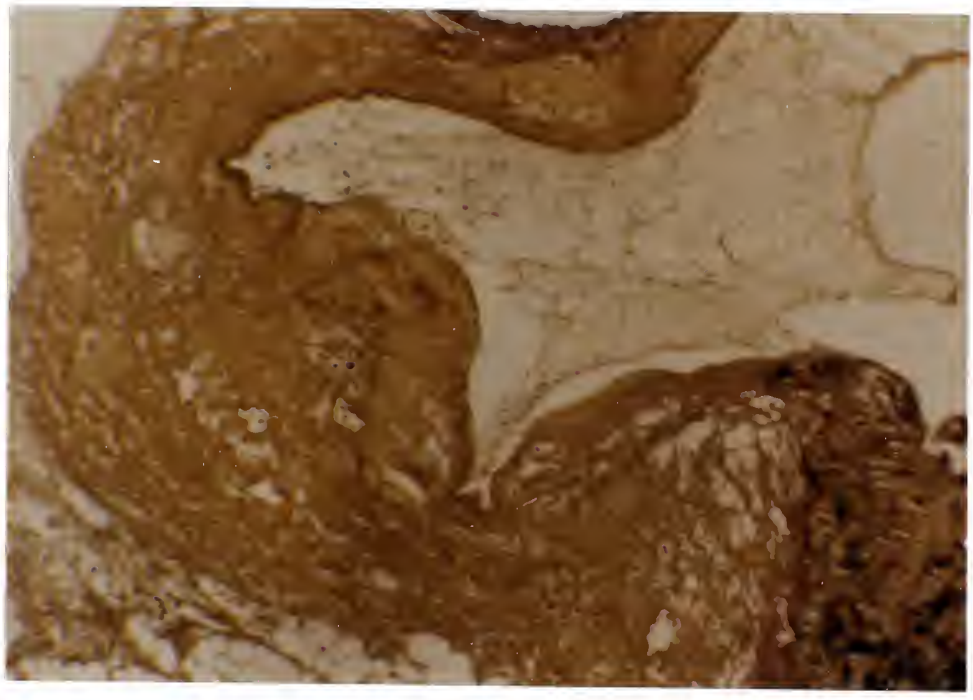


Figure 13 (#9, EVG) Site of rupture with disruption of the media and outward displacement of the adventitia. This segment of the aorta was in direct continuity wit the left ventricle.

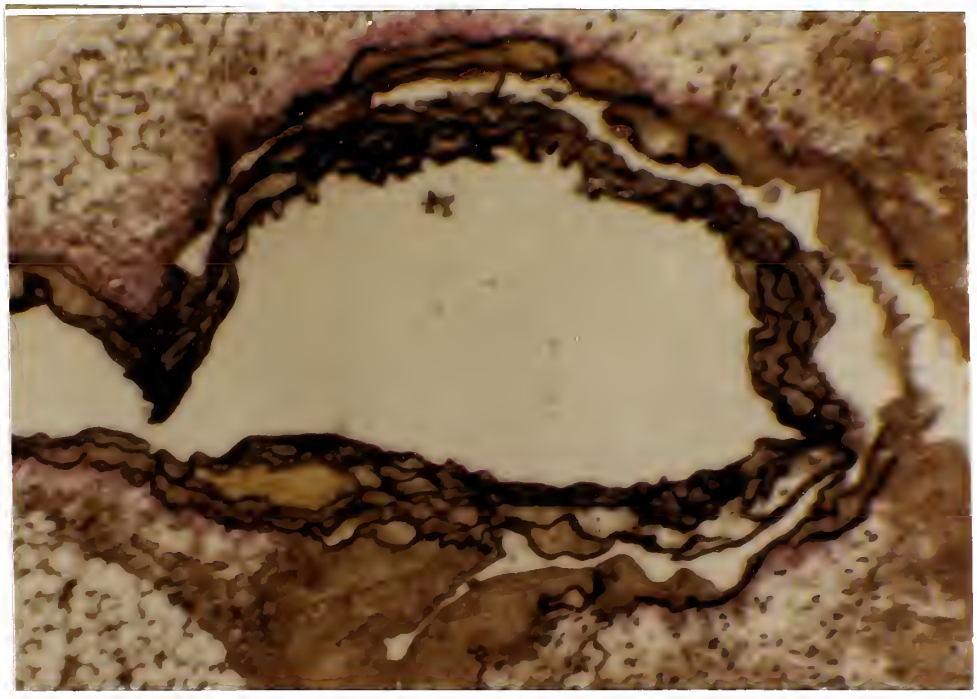


Figure 14A (#23, EVG) Section of aorta demonstrating dissection and rupture of the vessel wall with blood between elastic lamina, extramural blood and outward displacem of the adventitia.

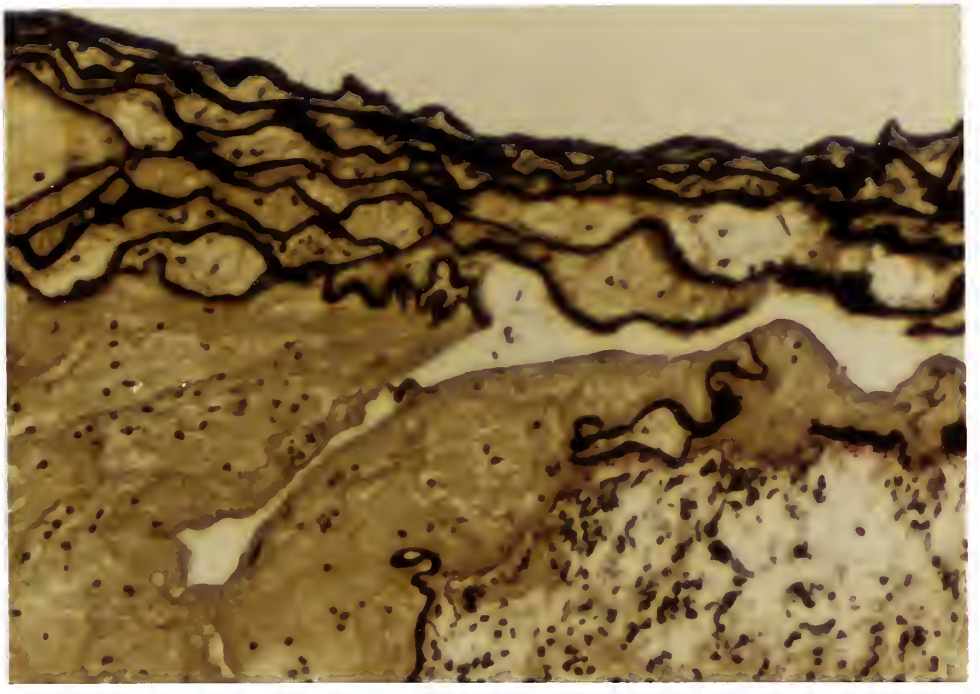


Figure 14B Magnification of section of 14A demonstrating disruption of elastic lamina and outward displacement of adventitia.

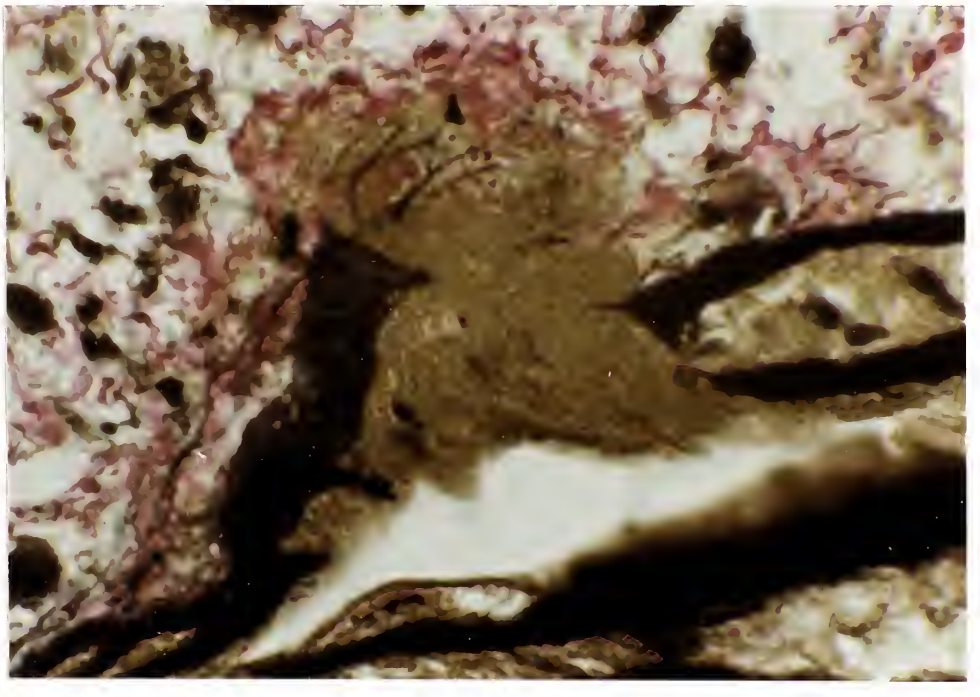


Figure 14C Magnification of 14A demonstrating focal site of disruption of elastic lamina.

Dose response curve for Hydrocortisone

Blotchy female mice were treated with 0.045, 0.15, 0.30 and 0.45 mg/ml of Hydrocortisone in their drinking water to derive a dose response curve. The data are presented in Tables VI - IX and two dose response curves are presented in figure 15. The first is based on data from Table VIII and represents the average length of survival of treated mice that died of aortic aneurysms within 21 days of beginning treatment. The second curve based on data presented in Table IX represents average survival of all mice up to 21 days. These curves illustrate two aspects of the effect of hydrocortisone on inducing aortic rupture: (a) The effect is dose dependent, and (b) the effect demonstrates a saturation point representing a minimum treatment time necessary to induce rupture. These are best demonstrated by the hyperbolic nature of the first curve.

Table VI

Data for derivation of dose response curve. (Mice sacrificed for angiography have been omitted.)

Mouse	Dose*	Age	Number	Day of Death	Gross Findings
Blotchy Female	0.045	2 mos	1	(21)	(alive)
			2	(21)	(alive)
			3	(21)	(alive)
			4	(21)	(alive)
			5	(21)	(alive)
Blotchy Female	0.15	2 mos	1	13	rupture
			2	19	rupture
			3	20	rupture
			4	20	rupture
			5	(21)	(alive)
Blotchy Female	0.30	2 mos	1	9	mutilated
			2	13	mutilated
			3	13	rupture
			4	15	rupture
			5	18	rupture
			6	19	rupture
Blotchy Female	0.45A	4 mos	1	12	rupture
		2 mos	2	11	mutilated
		12 mos	3	12	mutilated
		2 mos	4	13	rupture
		1 mos	5	13	rupture
		3 mos	6	14	rupture
		3 mos	7	9	rupture
		4 mos	8	15	rupture
	0.45B	3 mos	1	10	rupture
			2	13	rupture
			3	14	rupture
			4	15	rupture
			5	18	mutilated
			6	18	rupture
			7	18	rupture
			8	19	other
			9	19	rupture
			10	20	other

* Dose of hydrocortisone administered (mg/ml)

Table VII

Summary of experimental data.

Dose Hydrocortisone	0.045	0.15	0.30	0.45A	0.45B	0.45A&B
Aortic rupture	0	4	4	6	7	13
Mutilated	0	0	2	2	1	3
Other cause of death	0	0	0	0	2	2
Alive on day 21	5	1	0	0	0	0
Total	5	5	6	8	10	18

Table VIII

Average length of survival of treated mice that died of aortic rupture within 21 days, excluding those sacrificed. (The cause of death in the mutilated mice is assumed to be aortic rupture.)

Dose	0.045	0.15	0.30	0.45A	0.45B	0.45A&B
Aortic rupture (and mutilated)						
Number	0	4	6	8	8	16
Mean (days)	-	18.0	14.2	12.4	15.6	14.0
SD		3.4	3.6	1.8	3.2	3.0

Table IX

Average length of survival of treated mice dead and alive to 21 days, excluding those sacrificed.

Dose	0.045	0.15	0.30	0.45A	0.45B	0.45A&B
Cumulative days	105	93	87	99	164	263
Number	5	5	6	8	10	18
Mean (days)	21	18.6	14.5	12.4	16.4	14.6
SD	0	3.2	3.6	1.8	3.2	3.3

DOSE RESPONSE CURVE: AVERAGE LENGTH OF SURVIVAL TO AORTIC RUPTURE

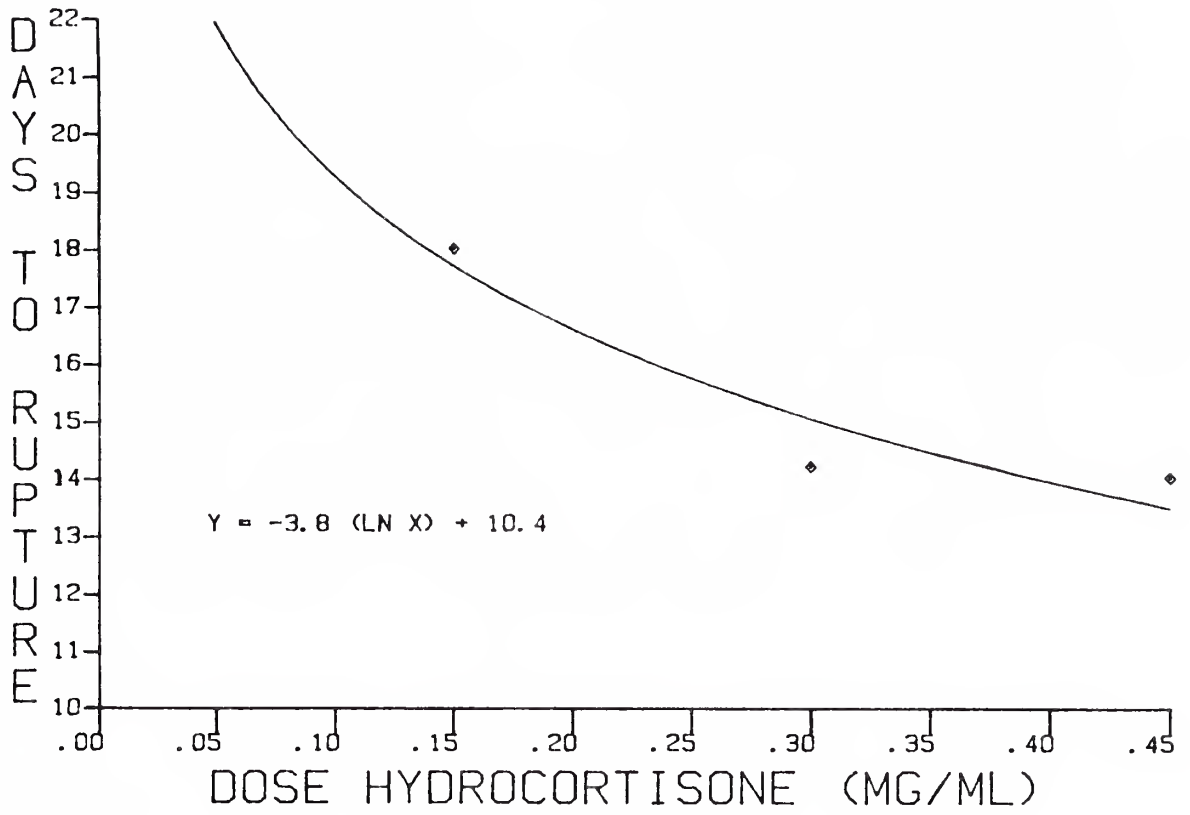


Figure 15A Average length of survival of treated mice that died of aortic rupture within 21 days. Data from table VIII. This table only includes mice that died of aortic rupture.

Standard Error=0.32

DOSE RESPONSE CURVE: AVERAGE SURVIVAL OF ALL MICE TO 21 DAYS

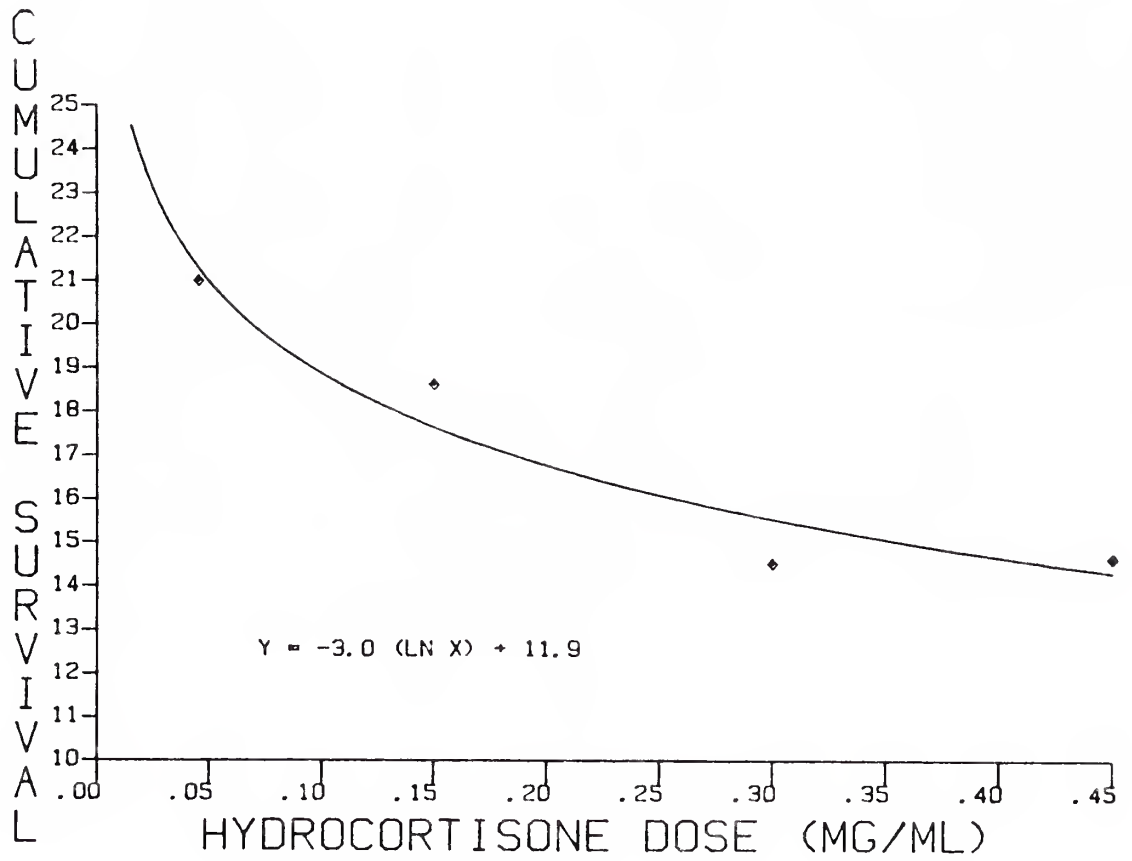


Figure 15B Average survival of all treated mice dead or alive to 21 days. Data from table IX. This table includes all mice treated.
Standard Error=0.267

Comparison of response to oral hydrocortisone in different phenotypes.

Three groups of mice were treated with 0.45 mg/ml of hydrocortisone to test for a variation in aortic rupture induction in different colonies and phenotypes. The three groups included Blotchy (Blo/+) and Black (+/+) female mice from our (Tilson) blotchy breeding colony and Black (+/+) C57BL/6J female mice from a pure strain maintained at Jackson Laboratories. The results are presented in Table X and summarized in Tables XI - XII. Note that all mice were 3 months old at the start of the experiment except for five of the Black (Tilson) which were two months old.

All of the mice were dead by day 32 and there where two principle findings at autopsy. A first group of mice usually had intrathoracic hemorrhage with accompanying hepatic pallor and no other abnormalities. A second group was found to have a thorax that was clear or had a trace of blood, a pale liver with no other sign of massive hemorrhage and the cause of death was indeterminate or appeared to involve dark, putrid gangrenous bowel of uncertain etiology. The general trend was for mice from our colony (Tilson) to die within 20 days of aortic rupture and after that with bowel necrosis. The average length of survival to aortic rupture in Blotchy (Tilson) and Black (Tilson) respectively was 15.6 ± 3.2 and 15.5 ± 5.4 days, the average length of

survival for all deaths was 16.4±3.2 and 17.5±7.9 days. (see Table XII)

(All deaths listed as mutilated have been assumed to be due to aortic rupture. The mutilation always involved the thoracic contents making diagnosis of aortic rupture impossible; but the abdominal contents were all intact and showed no sign of necrosis, thus this is a reasonable assumption.)

Unlike the mice from our breeding colony those from the Jackson Laboratories (C57BL/6J) predominantly died with bowel necrosis or another undetermined cause, (only two with aortic rupture) and also lived longer before death than the others, mean survival 19.0±5.9 days.

Seventy percent of the Blotchy (Tilson) mice died with aortic rupture. This result is similar to that of a previous experiment. Of the Black (Tilson) mice 64% died of aortic rupture and of the Black (C57BL/6) mice 20% died of aortic rupture. This is a sizable difference in rate of aortic rupture between the two groups of black mice. Chi-square evaluation of the difference in occurrence of aortic rupture in these two groups shows $P<0.05$, a statistically significant result. Comparison of the occurrence of aortic rupture in Blotchy and Black (Tilson) 70% and 64% respectively shows no significant difference.

Table X

Comparison of different phenotypes with a constant dose of hydrocortisone.

Mouse	Dose*	Age	Day of Death	Gross Findings
Blotchy Tilson (Blo/+)	0.45	3m	10--	
			13 ---	Intrathoracic rupture
			14 --	
			15--	
			18	Mutilated
			18	Intrathoracic rupture
			18	Intrathoracic rupture
			19	Pale liver, small amount of blood in thorax
			19	Intrathoracic rupture
			20	Pale liver; small amount of blood in thorax; dark, putrid bowel
Black Tilson (+/+)	0.45	3m	8	Mutilated
			11	Mutilated
			12--	
			18 --	
			20 ---	Intrathoracic rupture
			20 --	
			21--	
			24	Mutilated
			31	Pale liver; dark, putrid bowel
			31	Pale liver; dark, putrid bowel; intact saccular aortic arch aneurysm
		2m	6--	
			9 ---	Intrathoracic rupture
			15 --	
			19--	
			(22)	(Sacrificed day 22)
Black Jackson C57BL/6J	0.45	3m	13--	
			14 ---	Pale liver, small amount of blood and no clot in thorax
			15 --	
			15--	
			16	Intrathoracic rupture
			18	Intrathorcic rupture; dark, putrid bowel
			19--	
			23 ---	Dark, putrid bowel
			26 --	
			31--	

* Dose of hydrocortisone administered (mg/ml)

Table XI

Summary of experimental data

	Blotchy	Black*	C57BL/6J	Total
Aortic rupture	7	9	2	18
Mutilated	1	3	0	4
Other cause of death	2	2	8	12
Alive on day 32	0	0	0	0
Total	10	14	10	
% rupture	70%	64%	20%	

*sacrificed mouse excluded

Table XII

Average length of survival

	Blotchy	Black	C57BL/6J
All deaths			
Number	10	14	10
Mean (days)	16.4	17.5	19.0
SD	3.2	7.9	5.9
Aortic rupture (including mutilated mice-see text)			
Number	8	9	
Mean (days)	15.6	15.5	
SD	3.2	5.4	

DISCUSSION

Many of the side effects of steroid therapy are related to the steriod induced weakening of structural connective tissue. These experiments have studied one aspect of this: The induction of aortic rupture with oral Hydrocortisone in Blotchy mice.

Pathophysiology

On the most basic level these experiments have confirmed previous observations of the effects of high dose steroids on the aortas of turkeys and hamsters and extended these observations to mice. In these experiments 0.45 mg/ml of hydrocortisone in the drinking water precipitated aortic rupture in 70% percent of Blotchy females and in 64% percent of Black female littermates within 16 days. Rupture consistantly occurred in the thoracic aorta and, when location could be ascertained, appeared to involve the supravalvular portion of the ascending aorta. Some histological sections demonstrated clear transmural rupture, others demonstrated medial dissection with external rupture. All demonstrated disruption of medial elastin at the site of rupture. Angiography of treated mice demonstrated thoraco-abdominal and abdominal fusiform and saccular aneurysms with carotid and subclavian ectasia or dilatation. One mouse that

died with bowel necrosis had an intact saccular aneurysm of the aortic arch. Thus the type of large artery lesion induced by hydrocortisone is clear.

Etiology

Physiologically aortic rupture will result from a stress strain imbalance; stress due to systemic blood pressure and a lack of resistance to strain due to wall weakness. We did not measure systemic blood pressure changes with treatment in these mice but there is reason to believe that the blood pressure may have been elevated. Previous experiments have shown that cortisone acetate and methyl prednisolone will cause hypertension in rats (62, 65). Another investigator using the same treatment protocol used for these experiments noted that mice eventually doubled their daily water intake (39). This increased intake is due to the mild mineralocorticoid effect of the hydrocortisone and leads to increased vascular volume and possibly increased systemic pressure. Also notable was the marked cardiomegaly seen at thoracotomy when angiography was performed on mice with intact vascular systems. All this points to the development of systemic hypertension which would cause increased wall tension and contribute to aortic rupture.

Although increased stress is contributory, more important is the decreased resistance to strain induced by hydrocortisone. As already discussed vascular wall

integrity is principally maintained by collagen (31). Steroids have been shown to induce weakness and atrophy of collagen based connective tissues. This effect is mediated through selective diminution of new collagen synthesis possibly by direct influence of the steroid on transcription and translation (28, 66, 75, 87, 104). Prior experimental data points to this decreased anabolic effect as the principle cause of vessel wall weakness but the rupture of these aortas in two to three weeks time points to a concomitant rapid catabolism of soluble and insoluble aortic collagen. Does this represent the normal rate of collagen turnover which is no longer balanced by concurrent synthesis or is the actual rate of collagen catabolism increased? Two effects of steroids implicate an increased turnover rate as a contributer to the induction of aortic rupture, one direct and one indirect: Respectively, (A) steroids have been noted to increase collagenolytic activity in rat skin (53) and injured rabbit aortas (72) and; (B) and if blood pressure is elevated by the mineralocorticoid effects of hydrocortisone the necessary changes in tensile strength may promote aortic wall remodeling. In support of the latter possibility is the observation that there is increased collagenolytic activity in human aneurysmal aortas (13). This may represent a response to the increased wall tension created by the larger radius of the aneurysm. In either case, if this proposed induction of catabolism, as part of a response to stress, is not balanced by new structural protein synthesis then rupture

will quickly occur.

Dose dependency of rupture

A dose-response curve for the time course of rupture promotion is presented in figure 15. This curve is hyperbolic. Minimum effect (no rupture) is noted at the low dose end and the curve plateaus at the high dose end. This plateau at the high dose end suggests that the effect is saturable. There are two possible explanations for this plateau. The first is that the effect is receptor mediated and that at a certain dose saturation of this receptor occurs and additional drug will no longer effect the rate. In fact many of the effects of steroids in the body are known to be mediated by a cytosolic receptor. Receptors for specific steroids have been localized to the canine aortic media (52). This receptor may be responsible for the anabolic and catabolic effects of steroids. The second possibility is that once synthesis is reduced below a certain level the plateau represents the minimum amount of time necessary for collagen catabolism to weaken the aorta enough to allow rupture.

Blotchy Mouse Genetics

With the Blotchy mouse we have an animal model for genetic transmission of a trait for the development of aortic aneurysms. Not only is it clearly transmitted from generation to generation but in a very specific X-linked manner. Hemizygous males are pale and with a high degree of reliability (98%) develop and (90%) die from aortic aneurysms. Heterozygous females are mosaic in color and about 1/3 develop but few die from rupture of an aortic aneurysm. In light of the defect of copper metabolism shown to exist in these mice and the theory that it manifests itself by causing deficient activity of copper metalloenzymes, we thought the coat color might be a good marker for the effects of compounds on connective tissue strength and the development of aortic aneurysms. Having been made aware of the peculiar effects of an anti-angiogenesis regimen of heparin and hydrocortisone on mice and having noted in our own lab that the hemizygous male will turn darker when supplemented with copper, we were curious to see the effects of this regimen on mice known to have a defect in copper metabolism. We chose to use heterozygous female blotchy mice. To our surprise, in our mice treated with hydrocortisone we noted a remarkable number of deaths in two weeks. Nine of ten were dead; six of proven aortic rupture, and two more of probable rupture for a total of eight deaths attributable directly to the effects of

hydrocortisone, a highly statistically significant result ($P<0.001$). In comparison with previous studies of animals treated with steroids these mice ruptured after less time and in greater numbers. It is difficult to draw exact comparisons with these other experiments because they employed different steroids in different delivery systems at different dosages and in different animals. What is striking about this result is the high number of deaths after two weeks with the same regimen that another investigator had used in mice for thirty days to assess anti-angiogenesis effects, and that enough mice were alive at the end of thirty days for him to draw conclusions. We thought this was due to the effects of hydrocortisone on collagen metabolism compounding the copper metabolism defect presumably present in 1/2 of the heterozygous female's aortic smooth muscle cells. To prove this we had to show this effect of hydrocortisone was not as prominent in those mice thought not to be carrying the defect in copper metabolism. For this Black female (+/+) littermates of the Blotchy female (Blo/+) mice were used. They were all treated with 0.45 mg/ml of hydrocortisone and all demonstrated the same susceptability to the effects of the steroid. Seventy percent of the Blotchy ruptured in an average of 15.6 days and 64% of the Black ruptured in an average of 15.5 days. This raised the possibility that perhaps the Blotchy strain, at least those of our inbred colony may actually have a separate defect contributing to aortic weakness and predisposing to aneurysm

development. Perhaps this defect only weakened the aortic collagen mildly thus the Blotchy females which have adequate lysyl oxidase activity are not effected lethally. However when combined with the severely deficient lysyl oxidase activity in the hemizygous male due to the defect in copper metabolism a lethal combination arises and causes the frequent rupture characteristic of the Pale males.

Observations by other investigators are consistant with this hypothesis. Recall from Table I that while Brindled and Blotchy mice have a similar defect in copper metabolism 32% of (Blo/+) mice and 85% of (Blo/Blo) mice will develop aneurysms while 0% of (Br/+) and (Blo/Br) mice will (93). Skin lysyl oxidase activity of the Blotchy mouse does not respond as well to copper supplementation as that of the Brindled mouse and cytochrome C oxidase activity which responded quite well to copper supplementation in Brindled mice did not respond at all in Blotchy mice (16). Thus there must be another defect to explain the differences noted between these two strains of mice which have a similar defect in copper metabolism.

To support this hypothesis we acquired normal C57BL/6J female (+/+) mice that reportedly had no mottled relatives. We tested these mice for the vascular effects of hydrocortisone and found them to be less susceptible as compared to Black and Blotchy controls from our breeding colony. The C57BL/6J mice responded to hydrocortisone differently. Most of the mice from our breeding colony

Blotchy and Black died earlier deaths (within 18 to 20 days, mean 15.6±3.2 and 15.5±5.4 days) of aortic rupture and later deaths with bowel necrosis. Few of the C57BL/6J mice died of aortic rupture, most died later deaths with bowel necrosis or of undetermined causes (mean 19.0±5.9 days).

The common result noted in the mice from our colony which is dissimilar to that in C57BL/6J mice demonstrates that in our strain of Blotchy mice there is a predisposition to the development and rupture of aortic aneurysms distinct from the effects of the mottled locus. Assuming for the moment that this is a genetically transmitted trait with a single locus, which for the moment we will call "aneurysm", what classical modes of transmission are possible? The answer to this question is complex. If this gene is distinct from the classic mottled gene and if it is on a separate chromosome there should be independent assortment. If located on the same chromosome (the X-chromosome) there should be a certain amount of crossing over unless the two genes are located quite close to each other. In either case there would be two populations of Pale males; those that received the "aneurysm" gene and those that did not. There is not enough data available to adequately assess if there are two Pale male populations but the data in Table I reporting that 98% develop aneurysms suggests there are not two populations. Of importance here also is that the Blotchy mouse is an artificial population in that it is inbred. This selective inbreeding has been on the basis of coat color thus

the gene for "aneurysm" would have to be consistant with a pattern that could be bred for while selecting for the X-linked coat color allele. Taking all this into account, what modes of transmission are possible?

Autosomal recessive: For this to be true there would have to be identical defects in the genetic loci of the two autosomes. In a wild type setting where there is independent selection of mates a recessive trait like this would not be expressed in all the mice. However, in a selectively bred population such as ours; if no dominant normal alleles for this locus are in the gene pool the recessive trait will be maintained and expressed in all animals. One problem with this theory is that if this were a simple point mutation in a population that has been inbred for many years to produce many mice one would expect there to be a corrective mutation to add a dominant normal to the gene pool. Data to assess this are not available at this time.

Autosomal dominant: Again here the loci on both chromosomes would have to be identical. If there were any recessive/normal genes in the pool they would be expressed in an inbred population.

X-linked recessive and dominant: Unless present on both chromosomes these would give two separate pale male populations.

The only conclusion that can be drawn from this is that whatever the mutation, it must be present on both chromosomes to be maintained in this selectively bred population.

Implications for human beings.

In spite of the numerous deleterious side effects, steroid usage is widespread in medicine. Many people with severe autoimmune diseases, vasculitides, malignancies, et cetera are maintained on high doses of steroids to control symptoms or prevent or lessen disease progression. We have shown that, in mice predisposed to aneurysm development, steroids induce rapid and predictable aneurysm formation and rupture. The dose given mice treated with 0.45 mg/ml based on daily consumption of 7 milliliters of water by a 30 kg mouse is 0.105 mg/gm/day. The equivalent dose in a 70 kilogram man would be 7.4 gm/day. Obviously a hefty dose by any standard! Nevertheless this is the necessary dose in an experimental cancer anti-angiogenesis regimen (39). The investigator working with this regimen has since experimented with isomers of hydrocortisone which seem to have similar anti-angiogenetic properties without the glucocorticoid and anti-inflammatory effects of hydrocortisone. These were not available to us in sufficient quantities to test their effects on our model but it would be interesting to see if these do not cause aneurysms.

In light of the increasing evidence that aneurysmal disease in humans is a genetic trait (101), assessment of patients for aneurysmal disease before induction of and during prolonged steroid treatment would seem appropriate. This is especially important in patients with Marfan's

Syndrome who are known to have increased incidence of aortic dissection and patients with Ehlers-Danlos Syndrome who have weakness in other connective tissues besides the aorta and may be predisposed to rupture if steroids are prescribed. The only information available on the association of steroids and aneurysms is the results of drug trials in patients with Kawasaki's disease (59) and the increased incidence in pregnant woman (82), both discussed previously. There is no data relating the incidence of aneurysmal disease in patients on chronic steroid treatment.

Work is currently in progress to identify a biochemical marker for the aneurysmal trait and when identified will be useful to alert the physician to patients predisposed, especially when considering institution of chronic steroid therapy.

BIBLIOGRAPHY

1. Adinoff AD and Hollister JR: "Steroid-induced Fractures and Bone Loss in Patients with Asthma." N. Engl. J. Med. 309:265-8, 1983.

2. Alston JD et al.: "A Prospective Study of Hepatic and Skin Copper Levels in Patients with Abdominal Aortic Aneurysm." Surg. Forum 34:466-468, 1983.

3. Andrews EJ, White WJ and Bullock LP: "Spontaneous Aortic Aneurysms in Blotchy Mice." Am. J. Pathol. 78: 199-210, 1975.

4. Bartholomew JS and Anderson JC: "Investigation of Relationships between Collagens, Elastin and Proteoglycans in Bovine Thoracic Aorta by Immunofluorescence Techniques." Histochem. J. 15:1177-1190, 1983.

5. Beall CW et al.: "Aortic Rupture in Turkeys Induced by Diethylstilbestrol." Proc. Soc. Exp. Biol. Med. 113: 442-443, 1963.

6. Behrman RE and Vaughn VC: Nelson. Textbook of Pediatrics. Philadelphia:Saunders, 473, 1983.

7. Benson SC and LuValle PA: "Inhibition of Lysyl Oxidase and Prolyl Hydroxylase Activity in Glucocorticoid Treated Rats." Biochem. Biophys. Res. Commun. 99:557-562, 1981.

8. Beratis NG et al.: "64Cu Metabolism in Menkes' And Normal Cultured Skin Fibroblasts." Pediat. Res. 12: 699-702, 1978.

9. Booth BA et al.: "Steroid-induced Dermal Atrophy. Effects of Glucocorticosteroids on Collagen Metabolism in Human Skin Fibroblast Cultures." Int. J. Dermatol. 21:333-337, 1982.

10. Boucek RJ et al.: Modulation by Propranolol of the Lysyl Cross-links in Aortic Elastin and Collagen of the Aneurysm-prone Turkey. Biochem. Pharmacol. 32: 275-280, 1983.

11. Boucek RJ et al.: "The Marfan Syndrome: A Deficiency in Chemically Stable Collagen Cross-links." N. Engl. J. Med. 305:988-991, 1981.

12. Braunwald, Eugene: Heart Disease: A Textbook of Cardiovascular Medicine. Philadelphia: Saunders, 1542-1546, 1984.

13. Busuttil RW, Abou-Zamzam AM and Machleder HI: "Collagenase Activity of the Human Aorta - A Comparison of Patients With and Without Abdominal Aortic Aneurysms." Arch. Surg. 115:1373-1378, 1980.

14. Busuttil RW et al.: "Elastase Activity: The Role of Elastase in Aortic Aneurysm Formation." J. Surg. Res. 32:214-217, 1982.

15. Camakaris J et al.: "Copper Metabolism in Menkes' Syndrome and Mottled Mouse Mutants." In Inflammatory Diseases and Copper. Ed. Sorenson JRJ. Clifton, NJ: Humana Press, 85-96, 1982.

16. Camakaris J et al.: "Mutations in Humans and Animals which Affect Copper Metabolism." J. Inherited. Metab. Dis. 6 Suppl. 1:44-50, 1983.

17. Canalis E: "Effect of Glucocorticoids on Type I Collagen Synthesis, Alkaline Phosphatase Activity, and Deoxyribonucleic Acid Content in Cultured Rat Calvariae." Endocrinol. 112:931-939, 1983.

18. Cannon DJ, Casteel L and Read RC: "Abdominal Aortic Aneurysm, Leriche's Syndrome, Inguinal Herniation, and Smoking." Arch. Surg. 119:387-389, 1984.

19. Cannon DJ and Read RC: "Blood Elastolytic Activity in Patients with Aortic Aneurysm." Ann. Thorac. Surg. 34: 10-15, 1982.

20. Carnes WH: "Role of Copper in Connective Tissue Metabolism." Fed. Proc. 30:995-1000, 1971.

21. Clifton MA: "Familial Abdominal Aortic Aneurysms." Br. J. Surg. 64:765-766, 1977.

22. Cobb LM et al.: "Rupture of the Aorta Produced in the Hamster by Anti-ovulatory Progestogens." Nature 229:50-51, 1971.

23. Cooper JR, Bloom FE and Roth RH: The Biochemical Basis of Neuropharmacology, 4th Ed. New York: Oxford U. Press, 1982.

24. Counts DF, Rojas FJ and Cutroneo KR: "Glucocorticoids Decrease Prolyl Hydroxylase Activity without the Cellular Accumulation of Undehydroxylated Collagen." Mol. Pharmacol. 15:99-107, 1979.

25. Cox DR and Palmiter RD: "The Metallothionein-I Gene Maps to Mouse Chromosome 8: Implications for Human Menkes' Disease." Hum. Genet. 64:61-64, 1983.

26. Cutroneo KR, Costello D and Fuller GC: "Alteration of Proline Hydroxylase Activity by Glucocorticoids." Biochem. Pharmacol. 20:2797-2804, 1971.

27. Cutroneo KR and Counts DF: "Anti-inflammatory Steroids and Collagen Metabolism: Glucocorticoid-mediated Alterations of Prolyl Hydroxylase Activity and Collagen Synthesis." Mol. Pharmacol. 11:632-639, 1975.

28. Cutroneo KR, Rokowski R and Counts DF: "Glucocorticoids and Collagen Synthesis: Comparison of in vivo and Cell Culture Studies." Collagen Relat. Res. 1:557-568, 1981.

29. Danks DM: "Copper Deficiency in Humans." In Biological Roles of Copper. (CIBA Foundation Symposium, 79). Amsterdam:Excerpta Medica., 209-225, 1980.

30. Di Ferrante N et al.: "Lysyl Oxidase Deficiency in Ehler-Danlos Syndrome Type V." Connect. Tissue. Res. 3:49-53, 1975.

31. Dobrin PB, Baker WH and Gley WC: "Elastolyltic and Collagenolytic Studies of Arteries: Implications for the Mechanical Properties of Aneurysms." Arch. Surg. 119:405-409, 1984.

32. Doherty NS and Saarni H: "Stimulation by Hydrocortisone of the Rate of Collagen Synthesis in Cultured Fibroblasts." J. Pharm. Pharmac. 28:656-657, 1976.

33. Elefteriades J, Panjabi MJ and Tilson, MD: "Reduced Tensile Strength of Skin from the Spontaneously Aneurysm-prone Blotchy Mouse." Surg. Forum 33:58-60, 1982.

34. Estes Jr. JE: "Abdominal Aortic Aneurysm: A Study of One Hundred and Two Cases." Circulation 2:258-264, 1950.

35. Evans GW and Reis BL: "Impaired Copper Homeostasis in Neonatal Male and Adult Female Brindled Mice." J. Nutr. 108:554-560, 1978.

36. Eyre DR: "Collagen: Molecular Diversity in the Body's Protein Scaffold." Science 207:1315-1322, 1980.

37. Eyre DR, Paz MA and Gallop PM: "Cross-linking in Collagen and Elastin." Annu. Rev. Biochem. 53:717-748, 1984.

38. Fischer GM and Swain ML: "Influence of Contraceptive and Other Sex Steroids on Aortic Collagen and Elastin." Exp. Mol. Pathol. 33:15-24, 1980.

39. Folkman J et al.: "Angiogenesis Inhibition and Tumor Regression Caused by Heparin or a Heparin Fragment in the Presence of Cortisone." Science 221:719-725, 1983.

40. Frenkel JK: "Dissecting Aneurysms of the Aorta and Pancreatic Islet Cell Hyperplasia with Diabetes in Corticosteroid- and Chlorothiazid-treated Hamsters." Progr. Exp. Tumor Res. 16:300-324, 1972.

41. Frenkel JK, Rasmussen P and Smith OD: "Synergism of Cortisol and Desoxycorticosterone in the Production of Dissecting Aortic Aneurysms in Hamsters." Fed. Proc. 18: 477, 1959.

42. Fujimoto D: "Evidence for Natural Existence of Pyridinoline Cross-link in Collagen." Biochem. Biophys. Res. Commun. 93:948-953, 1980.

43. Fujimoto D, Akiba K and Nakamura N: "Isolation and Characterization of a Fluorescent Material in Bovine Achilles Tendon Collagen." Biochem. Biophys. Res. Commun. 76:1124-1129, 1977.

44. Glagov S and Zarins CK: "Pathophysiology of Aneurysm Formation." In Aneurysms. Ed. Kerstein MD, Moulder PV, and Webb WR. Baltimore:Williams & Wilkins, 1-18, 1983.

45. Goldfischer S et al.: "The Infrastructure of Aortic Elastic Fibers." Tissue Cell 15:429-435, 1983.

46. Graham LM, Zelenock GB et al.: "Clinical Significance of Atherosclerotic Femoral Artery Aneurysms." Arch. Surg. 115:502-507, 1980.

47. Gresham GA and Howard AN: "Aortic Rupture in the Turkey." J. Atheroscler. Res. 1:75-80, 1961.

48. Harris ED: "Copper-induced Activation of Aortic Lysyl Oxidase in Vivo." Proc. Natl. Acad. Sci. 73:371-374, 1976.

49. Harris ED et al.: "Copper and the Synthesis of Elastin and Collagen." In Biological Roles of Copper. (CIBA Foundation Symposium, 79). Amsterdam:Excerpta Medica, 163-182, 1980.

50. Holstein TJ et al.: "Effect of Altered Copper Metabolism Induced by Mottled Alleles and Diet on Mouse Tyrosinase." Proc. Soc. Exp. Biol. Med. 162:264-8, 1979.

51. Hornebeck W, Adnet JJ and Robert L: "Age Dependent Variation of Elastin and Elastase in Aorta and Human

Breast Cancers." Exp. Gerontol. 13:293-8, 1978.

52. Horwitz KB and Horwitz LD: "Canine Vascular Tissues are Targets for Androgens, Estrogens, Progestins, and Glucocorticoids." J. Clin. Invest. 69:750-758, 1982.

53. Houck JC et al.: "Induction of Collagenolytic and Proteolytic Activities by Anti-inflammatory Drugs in the Skin and Fibroblast." Biochem. Pharmacol. 17:2081-2090, 1968.

54. Hunt DM: "A Study of Copper Treatment and Tissue Copper Levels in the Murine Congenital Copper Deficiency, Mottled." Life Sciences 19:1913-1920, 1976.

55. Hunt DM: "Primary Defect in Copper Transport Underlies Mottled Mutants in the Mouse." Nature 249:852-854, 1974.

56. Hunt DM and Johnson DR: "An Inherited Deficiency in Noradrenaline Biosynthesis in the Brindled Mouse." J. Neurochem. 19:2811-2819, 1972.

57. Hunt DM and Johnson DR: "Aromatic Amino Acid Metabolism in Brindled Mice and Viable-brindled, Two Alleles at the Mottled Locus in the Mouse." Biochem. Genet. 6:31-40, 1972.

58. Jarvelainen H, Halme T and Ronnemaa T: "Effect of Cortisol on the Proliferation and Protein Synthesis of Human Aortic Smooth Muscle Cells in Culture." Acta Med. Scand. [suppl] 660:114-122, 1982.

59. Kato H, Koike S and Yokoyama T: "Kawasaki Disease: Effect of Treatment on Coronary Artery Involvement." Pediatrics 63:175-179, 1979.

60. Kivirikko KI et al.: "Studies with 14C-Proline on the Action of Cortisone on the Metabolism of Collagen in the Rat." Biochem. Pharmacol. 14:1445-1451, 1965.

61. Kleinman M and Gross AE: "Achilles Tendon Rupture Following Steroid Injection. A Report of Three Cases." J. Bone Joint Surg. 65A:1345-1347, 1983.

62. Knowlton AI et al.: "Induction of Arterial Hypertension in Normal and Adrenalectomized Rats Given Cortisone Acetate." J. Exp. Med. 96:187-205, 1952.

63. Korner A and Pawelek J: "Mammalian Tyrosinase Catalyzes Three Reactions in the Biosynthesis of Melanin." Science 217:1163-5, 1982.

64. Krakoff L, Nicolis G and Amsel B: "Pathogenesis of

Hypertension in Cushing's Syndrome." Am. J. Med. 58: 216-220, 1975.

65. Krakoff LR, Selvadurai R and Sutter E: "Effect of Methylprednisolone upon Arterial Pressure and the Renin Angiotensin System in the Rat." Am. J. Physiol. 228: 613-617, 1975.

66. Kruse NJ et al.: "Inhibitory Effects of Glucocorticoids on Collagen Synthesis by Mouse Sponge Granulomas and Granuloma Fibroblasts in Culture." Biochem. Biophys. Acta. 540:101-116, 1978.

67. Kuivaniemi H et al.: "Abnormal Copper Metabolism and Deficient Lysyl Oxidase Activity in a Heritable Connective Tissue Disorder." J. Clin. Invest. 69:730-733, 1982.

68. Lee HB: "Avulsion and Rupture of the Tendo Calcaneus After Injection of Hydrocortisone." Br. Med. J. 2:395, 1957.

69. Leitman DC, Benson SC and Johnson LK: "Glucocorticoids Stimulate Collagen and Noncollagen Protein Synthesis in Cultured Vascular Smooth Muscle Cells." J. Cell Biol. 98:541-549, 1984.

70. Lemp MA: "Cornea and Sclera." Arch. Ophthalmol. 94: 473-490, 1976.

71. Longenecker JP, Kilty LA and Johnson LK: "Glucocorticoid Inhibition of Vascular Smooth Muscle Cell Proliferation: Influence of Homologous Extracellular Matrix and Serum Mitogens." J. Cell Biol. 98:534-540, 1984.

72. Manthrope R: "Glucocorticosteroid Effect on Vascular Connective Tissue." Acta. Endocrinol. [suppl] 259: 1-40, 1983.

73. Martin P: "On Abdominal Aortic Aneurysms." J. Cardiovasc. Surg. 19:597-599, 1978.

74. Neil-Dwyer G et al.: "Collagen Deficiency and Ruptured Cerebral Aneurysms: A Clinical and Biochemical Study." J. Neurosurg. 59:16-20, 1983.

75. Newman RA and Cutroneo KR: "Glucocorticoids Selectively Decrease the Synthesis of Hydroxylated Collagen Peptides." Mol. Pharmacol. 14:185-198, 1978.

76. Oikarinen A: "Effect of Cortisol Acetate on Collagen Biosynthesis and on the Activities of Prolyl Hydroxylase, Lysyl Hydroxylase, Collagen Galactosyltransferase

and Collagen Glucosyltransferas in Chick-embryo Tendon Cells." Biochem. J. 164:533-539, 1977.

77. Packman S et al.: "Cadmium, Zinc, and Copper Metabolism in the Mottled Mouse, An Animal Model for Menkes' Kinky Hair Syndrome." J. Inorg. Biochem. 19:203-211, 1983.

78. Peltonen L et al.: "Alterations in Copper and Collagen Metabolism in the Menkes Syndrome and a New Subtype of the Ehlers-Danlos Syndrome." Biochemistry 22:6156-6163, 1983.

79. Pinnell SR and Martin GR: "The Cross-linking of Collagen and Elastin: Enzymatic Conversion of Lysine in Peptide Linkage to alpha-Aminoadipic-delta-Semi-aldehyde (Allysine) by an Extract from Bone." Proc. Natl. Acad. Sci. 61:708-716, 1968.

80. Prins HW and Van den Hamer CJA: "Primary Biochemical Defect in Copper Metabolism in Mice with a Recessive X-Linked Mutation Analogous to Menkes' Disease in Man." J. Inorg. Biochem. 10:19-27, 1979.

81. Rayton JK and Harris ED: "Induction of Lysyl Oxidase with Copper: Properties of and IN VITRO System." J. Biol. Chem. 254:621-626, 1979

82. Roberts WC: "Aortic Dissection: Anatomy, consequences, and causes." Am. Heart J. 101:195-214, 1981.

83. Rokowski RJ, Sheehy J and Cutroneo KR: "Glucocorticoid-mediated Selective Reduction of Functioning Collagen Messenger Ribonucleic Acid." Arch. Biochem. Biophys. 210:74-81, 1981.

84. Rowe DW et al.: "A Sex Linked Defect in Crosslinking of Collagen and Elastin Associated with the Mottled Locus in Mice." J. Exp. Med. 139:180-192, 1974.

85. Rowe DW et al: "Decreased Lysyl Oxidase Activity in the Aneurysm-prone Mottled Mouse." J. Biol. Chem. 252: 939-942, 1977.

86. Royce PM, Camakaris J and Danks DM: "Reduced Lysyl Oxidase Activity in SKin Fibroblasts from Patients with Menkes' Syndrome." Biochem. J. 192:579-586, 1980.

87. Russell SB, Russell JD and Trupin KM: "Collagen Synthesis in Human Fibroblasts: Effects of Ascorbic Acid and Regulation by Hydrocortisone." J. Cell. Physiol. 109:121-131, 1981.

88. Saarni H and Tammi M: "Time and Concentration Dependence of the Action of Cortisol on Fibroblasts In

Vitro." Biochim. Biophys. Acta 540:117-126, 1978.

89. Sandberg LB, Soskel NT and Leslie JG: "Elastin Structure, Biosynthesis, and Relation to Disease States." N. Engl. J. Med. 304:566-579, 1981.

90. Schlatmann TJM and Becker AE: "Histologic Changes in the Normal Aging Aorta: Implications for Dissecting Aortic Aneurysm." Am. J. Cardiol. 39:13-20, 1977.

91. Shamberger RJ: "Serum Ceruloplasmin and Copper Levels in Normals and in Patients with Aortic Aneurysms." Fed. Proc. 42:528, 1983.

92. Silberberg, R: "Epiphyseal Growth and Osteoarthritis in Blothcy Mice." Exp. Cell Biol. 45:1-8, 1977.

93. Silvers WK: The Coat Colors of Mice. A Model for Mammalian Gene Action and Interaction. New York: Springer-Verlag, 1979.

94. Smith QT: "Radioactivity of Hydroxyproline From Urine and Collagens of Normal and Cortisone-treated Rats." Biochem. Pharmacol. 16:2171-2179, 1967.

95. Starcher B et al.: "Abnormal Cellular Copper Metabolism in the Blotchy Mouse." J. Nutr. 108:1229-1233, 1978.

96. Steffee CH and Snell KC: "Dissecting Aortic Aneurysm in Hamsters Treated with Cortisone Acetate." Proc. Soc. Exp. Biol. Med. 90:712-714, 1955.

97. Sumner DS, Hokanson DE and Strandness DE: "Stress-strain Characteristics and Collagen-Elastin Content of Abdominal Aortic Aneurysms." Surg. Gynecol. Obstet. 130:459-466, 1970.

98. Tilson MD: "Decreased Hepatic Copper Levels: A Possible Chemical Marker for the Pathognesis of Aortic Aneurysms in Man." Arch. Surg. 117:1212-1213, 1982.

99. Tilson MD and Dang C: "Generalized Arteriomegaly: A Possible Predisposition to the Formation of Abdominal Aortic Aneurysms." Arch. Surg. 116:1030-1032, 1981.

100. Tilson MD and Davis G: "Deficiencies of Copper and a Compound with Ion-exchange Characteristics of Pyridinoline in Skin from Patients with Abdominal Aortic Aneurysms." Surgery 94:134-141, 1983.

101. Tilson MD and Seashore MR: "Fifty Families With Abdominal Aortic Aneurysms in Two or More First-Order Relatives." Am. J. Surg. 147:551-553, 1984.

102. Tilson MD and Seashore MR: "Human Genetics of the Abdominal Aortic Aneurysms." Surg. Gynecol. Obstet. 158:129-132, 1982.

103. Tilson MD and Stansel HD: "Differences in Results for Aneurysms versus Occlusive Disease After Bifurcations Grafts: Results of 100 Elective Grafts." Arch. Surg. 115:1173-1175, 1980.

104. Verbruggen LA et al.: "Relationship of Cell Growth to Collagen Synthesis in Glucocorticoid Treated A/J and C57BL6/J Neonatal Mouse Dermal Fibroblasts." Scand. J. Rheumatol. 12:360-366, 1983.

105. Wenk G and Suzuki K: "Congenital Copper Deficiency: Copper Therapy and Dopamine-B-Hydroxylase Activity in the Mottled (Brindled) Mouse." J. Neurochem. 41: 1648-52, 1983.

106. Wolinsky, H and Glagov S.: "Nature of Species Differences in the Medial Distribution of Aortic Vasa Vasorum in Mammals." Circ. Res. 20:409-21, 1967.

107. Zarins CK and Glagov S: "Aneurysms and Obstructive Plaques. Differing Local Responses to Atherosclerosis." In Aneurysms. Diagnosis and Treatment. Ed. Bergan JJ and Yao JST. New York: Grune & Stratton, 61-82, 1982.

YALE MEDICAL LIBRARY

Manuscript Theses

Unpublished theses submitted for the Master's and Doctor's degrees and deposited in the Yale Medical Library are to be used only with due regard to the rights of the authors. Bibliographical references may be noted, but passages must not be copied without permission of the authors, and without proper credit being given in subsequent written or published work.

This thesis by has been used by the following persons, whose signatures attest their acceptance of the above restrictions.

NAME AND ADDRESS	DATE